# (19) World Intellectual Property Organization

International Bureau





(43) International Publication Date 12 April 2007 (12.04.2007)

(51) International Patent Classification: C12Q 1/68 (2006.01)

(21) International Application Number:

PCT/EP2006/010132

(22) International Filing Date:

29 September 2006 (29.09.2006)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

05109025.6

29 September 2005 (29.09.2005) EP

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(10) International Publication Number WO 2007/039319 A2

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

#### **Published:**

without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANALYTICAL DEVICE FOR RAPID IDENTIFICATION OF PATHOGENS

(57) Abstract: The present invention provides an analytical device, especially a DNA microarray, for identification and characterisation of microorganisms in a sample or clinical specimen. Furthermore, it provides for a method for rapid identification and strain profiling of different microbial species in a sample or clinical specimen, especially in a blood culture, utilizing said analytical device.

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### **Analytical device for Rapid Identification of Pathogens**

The present invention provides an analytical device, especially a DNA microarray, for identification and characterisation of microorganisms in a sample or clinical specimen. Furthermore, it provides for a method for rapid identification and strain profiling of different microbial species in a sample or clinical specimen, especially in a blood culture, utilizing said analytical device.

## Background

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Isolation, identification and characterisation of bacteria and fungi from such diverse samples like food, environmental samples, clinical specimens, and veterinary samples is still a challenge for today's analytical laboratories. This is due to the fact that generally the identification of microorganisms includes three steps: (a) enrichment of microorganisms by culture, (b) subculture on solid media (preparation of a pure culture), and (c) performing a set of biochemical reactions specific for a particular pathogen. All these steps are dependent on the bacterial growth (slow), they are poorly automated (lot of manual work), and complex (require well educated personal).

Isolation, identification and characterisation of bacteria and fungi from clinical specimens is a main task of microbiological routine diagnostics. In fact, microorganisms are ubiquitous in certain areas of the human body. For this reason isolation and identification of pathogenic bacteria from clinical material and discrimination of specific pathogens from contaminations with indigenous or environmentally encountered microorganisms is a requirement for the correct diagnosis of infectious diseases. Additionally, accurate identification of antibiotic resistance and particular virulence factors provide important information enabling the clinician to choose effective antimicrobial therapy.

In the course of infection, many specimen types can be used for direct identification of the pathogens. These include, but are not limited to, liquor in the course of bacterial meningitis, sputum from patients with bacterial pneumonia, urine in the course of upper and lower urinary tract infections, punktate from sites of deep purulent infections (such as abscess, phlegmone, lung emphysema and septic arthritis), stool from patients with gastrointestinal tract infections, pus, swabs or wound fluid from purulent infections of the skin and wounds. Sometimes, bacteria

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are represented in the specimen only in minor numbers, thus, indirect identification of pathogens after culture of specimens in liquid media is employed. Important examples are enrichment cultures of food samples during outbreaks of food borne infections and blood cultures for diagnosis of bloodstream infections.

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The invasion of the bloodstream by microorganisms, especially bacteremia and fungemia, represents one of the most serious consequences of infections and is a high ranked cause of death (Mylotte, J.M. and Tayara, A., Eur. Clin. Microbiol. Infect. Dis. 19:157-163 (2000); Reimer, L.G. et al., Clin. Microbiol. Rev. 10:444-465 (1997)). Bacteremia is the means by which local infections spread hematogenously to distant organs. This hematogenous dissemination of bacteria is part of the pathophysiology of, e.g., meningitis and endocarditis, Pott's disease and many other forms of osteomyelitis. In the hospital, indwelling catheters are a frequent cause of bacteremia and subsequent nosocomial infections, since they provide a means by which bacteria normally found on the skin can enter the bloodstream. Other causes of bacteremia include dental procedures, urinary tract infections, intravenous drug use, and colorectal cancer.

Systemic fungal infection is becoming more and more common in modern hospitals. The most common fungal infections are candidiasis and aspergillosis, but other such Histoplasmosis, systemic fungal infections as Blastomycosis, Coccidioidomycosis and Cryptococcosis are also of increasing relevance. Systemic fungal infections in hospitals are commonly seen in immune compromised patients and - like bacteremia - in patients with indewelling catheters. Due to underlying serious illnesses and possible resistance of the pathogens to antifungal agents, patients with systemic fungal infections often have poor clinical outcomes. Infections due to Candida species are the fourth most important cause of nosocomial bloodstream infection.

Bacteremia is operationally defined as the presence of viable bacteria as evidenced by positive blood cultures. Fungemia is similarly defined as the presence of viable fungi as evidenced by positive blood cultures. When bacteremia or fungemia occurs in the presence of systemic symptoms (such as fever or chills) the condition is designated as sepsis; and in the setting of more severe disturbances of

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temperature, respiration, heart rate or white blood cell count, is characterised as systemic inflammatory response syndrome (SIRS).

Many septic episodes are nosocomial and often due to microorganisms with increased and multiple antimicrobial resistance. Staphylococcus aureus, Escherichia Coaqulase-negative staphylococci (CoNS), Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus spp., Streptococcus spp., Candida albicans and Enterobacter cloacae are the most frequent etiological agents of bacteremia and fungemia in Europe (Decousser, J. W. et al., J. Antimicrob. Chemother. 51:1214-22 (2003); Lyytikainen, O. et al., Clin. Infect. Dis. 35:314-9 (2002); Reacher, M.H. et al., BMJ 320:213-6 (2000); Rosenthal Kreuberger, E.J., Int. J. Antimicrob. Agents 24:196-8 (2004)) and the USA (Bourbeau, P.P. and Pohlman, J.K., J. Clin. Microbiol. 39:2079-82 (2001); Reimer, L.G. et al., Clin. Microbiol. Rev. 10:444-65 (1997); Reisner, L.G. et al., J. Clin. Microbiol. 37:2024-6 (1999); Wilson, M.L. et al., J. Clin. Microbiol. 37:1709-13 (1999)).

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Nosocomial bacteremia and especially sepsis require an immediate antibiotic therapy, even when the causative bacteria are still unknown. Thus, said therapy has to be performed as empirical initial therapy (Rello, J. et al., Intensive Care Med. 20:94-98 (1994)), which covers the complete spectrum of relevant pathogens. However, the increase of bacterial resistance lowers the chance of success for such empirical antibiotic treatments considerably (Mylotte, J.M. and Tayara, A., Eur. Clin. Microbiol. Infect. Dis. 19:157-163 (2000); Weinstein, M.P. et al., Clin. Infect. Dis. 24:584-602 (1997)). This primary therapy can only be replaced by a specific treatment after a thorough microbial diagnosis which usually takes 76-120 h (Bourbeau, P.P. and Pohlman, J.K., J. Clin. Microbiol. 39:2079-2082 (2001)). A fast track diagnosis which shortens this lag time would increase the chance of therapy success.

Rapid and reliable detection of bloodstream infections, including characterisation of the pathogen to the species level and determination of its antibiotic susceptibility pattern, is crucial for several reasons: (i) Appropriate antimicrobial agents can be selected, and thus, unnecessary treatment with ineffective antibiotics can be avoided; (ii) the prognosis of the patients can be improved; (iii) the acquisition of resistances in pathogens may be decelerated and (iv) expenditures on antimicrobials and overall hospital costs can be reduced (Barenfanger, J. et al., J.

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Clin. Microbiol. 37:1415-8 (1999); Doern, G.V. et al., J. Clin. Microbiol. 32:1757-62 (1994); Trenholme, G.M. et al., J. Clin. Microbiol. 27:1342-5 (1989); Wheeler, A.P. and Bernard, G.R., N. Engl. J. Med. 340:207-14 (1999)). Therefore, there is a strong need for rapid tests for specific and sensitive identification of bacteria and pathogenic fungi directly from blood cultures.

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The diagnosis of bacteremia commonly relies on blood cultures where the growth of microorganisms is continuously monitored by automated devices (James, P.A. and Al-Shafi, K.M., J. Clin. Pathol. 53:231-233 (2000); Reisner, B.S. and Woods, G.L., J. Clin. Microbiol. 37:2024-2026 (1999); Wilson, M.L. et al., J. Clin. Microbiol 37:1709-1713 (1999)). Although such continuous-reading and computed systems decrease the time for detection of positive blood cultures, definitive pathogen identification from positive blood cultures still requires traditional Gram-staining, sub-culturing and susceptibility testing, delaying the identification of pathogens for one to three days (Levi, K. and Towner, K.J., J. Clin. Microbiol. 41:3890-3892 (2003); Oliveira, K. et al., J. Clin. Microbiol. 41:889-891 (2003); Oliveira, K. et al., J. Clin. Microbiol. 40:247-251 (2002); Tan, T.Y. et al., J. Clin. Microbiol. 39:4529-4531 (2001)). The subculture procedure with subsequent species identification and determination of antibiotic resistance is time-consuming and elaborate. The biochemical and immunological assays like testing with coagulase, nuclease or latex agglutination are not always reliable. Antigenic and biochemical variations of bacteria grown in blood culture, inhibitory action of blood culture medium components as well as the presence of more than one microbial species may mislead data interpretation.

Staphylococci are the most important and frequent group of pathogens growing in blood culture, responsible for 30% to more than 50% of all bacteremia events (James, P.A. and Al-Shafi, K.M., J. Clin. Pathol. 53:231-233 (2000); Reisner, B.S. and Woods, G.L., J. Clin. Microbiol. 37:2024-2026 (1999); Velasco, E. et al., Sao Paulo Med. J. 118:131-138 (2000)) with a mortality rate ranging from 13 to 50% (McClelland, R.S. et al., Arch. Intern. Med. 159:1244-1247 (1999); Rello, J. et al., Intensive Care Med. 20:94-98 (1994); Weinstein, M.P. et al., Clin. Infect. Dis. 24:584-602 (1997)). The emergence of *S. aureus* strains with multiple resistance to antibiotics makes empirical therapy prone to fail (Tan, T.Y. et al., J. Clin. Microbiol. 39:4529-4531 (2001)). *S. aureus* is generally regarded as a virulent

pathogen, whereas CoNS are either considered as a cause of catheter-associated nosocomial bacteremia or, more frequently, as blood culture contamination. Thus, a sub-genus identification of gram-positive cocci in clusters (CPCC) is of great clinical significance (Oliveira, K. et al., J. Clin. Microbiol. 41:889-891 (2003)).

Methods used up to date for direct identification of *S. aureus* growing in blood culture bottles include biochemical tests, like detection of thermostable nuclease or tube coagulase test, or commercial antibody-based kits connected with the disadvantages listed above.

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Besides S. aureus and coagulase-negative staphylococci, E. coli, Klebsiella spp., Enterobacter spp., Proteus spp., Pseudomonas aeruginosa, Streptococcus pneumoniae, beta hemolytic Streptococci and Enterococcus spp. belong to the most frequent reported pathogens causing bacteremia (Reimer, L.G. et al., Clin. Microbiol. Rev., 10:444-65 (1997); Reacher, M.H. et al., BMJ, 320:213-6 (2000); Lyytikainen, O. et al., Clin. Infect. Dis., 35:e14-9 (2002)) In order to reduce the time needed for identification and susceptibility testing, the possibility of combining an automated blood culture system with an automated identification and susceptibility testing system by direct inoculation from positive blood cultures has been studied for gram-positive cocci as well as for gram-negative rods by several groups of investigators, but with varying success (Reimer, L.G. et al., Clin. Microbiol. Rev., 10:444-65 (1997); Hansen, D.S. et al., Clin. Microbiol. Infect., 8:38-44 (2002); Ling, T.K. et al., J. Clin. Microbiol., 41:4705-7 (2003); Funke, G. and Funke-Kissling, P., J. Clin. Microbiol., 42:1466-70 (2004)). Although the authors saw some potential of the combined system to allow the agar isolation step to be skipped, the system is hampered by the fact that (i) the blood culture sample has to undergo a time-consuming separation procedure for the enrichment of bacterial cells, (ii) the identification rate varies depending on the employed identification system and (iii) the performance is not equally good for gram-negative and gram-positive pathogens (Reimer, L.G. et al., Clin. Microbiol. Rev., 10:444-65 (1997); Ling, T.K. et al., J. Clin. Microbiol., 41:4705-7 (2003); Funke, G. and Funke-Kissling, P., J. Clin. Microbiol., 42:1466-70 (2004)).

Considerable progress was made using nucleic acid-based methods for the identification and genotyping of bacteria or fungi in blood specimens. Assays employing ribosomal RNA-based oligonucleotide probes like fluorescence *in situ* 

hybridisation (FISH) (Chapin, K. and Musgnug, M., J. Clin. Microbiol. 41:4324-7 (2003); Jansen, G.J. et al., J. Clin. Microbiol. 38:814-7 (2000); Kempf, V.A. et al., J. Clin. Microbiol. 38:830-8 (2000); Oliveira, K. et al., J. Clin. Microbiol. 41-889-91 (2003)) or microarrays (Anthony, R.M. et al., J. Clin. Microbiol. 38:781-8 (2000); Marlowe, E.M. et al., J. Clin. Microbiol. 41:5127-33 (2003); Sogaard, M. et al., J. Clin. Microbiol., 43:1947-9 (2005)) provide for rapid species identification in blood cultures. However, methods solely based on ribosomal RNA probes allow species identification only, and do not provide information on antibiotic susceptibility and other strain specific characteristics (e.g. virulence genes). For the molecular detection of antibiotic resistances in staphylococci, several multiplex PCR-based assays were described (Martineau, F. et al., Antimicrob. Agents Chemother. 44:231-8 (2000); Shrestha, N.K. et al., Approved standard M2-4A, Villanova, PA (1990); Strommenger, B.C. et al. J. Clin. Microbiol. 41:4089-94; Tan, T.Y. et al., J. Clin. Microbiol. 39:4529-31 (2001)). Several groups have successfully identified S. aureus and more specifically methicillin-resistant S. aureus strains (MRSA) from blood cultures by using DNA probes (Levi, K. and Towner, K.J., J. Clin. Microbiol. 41:3890-3892 (2003); Poulsen, A.B. et al., J. Antimicrob. Chemother. 51:419-421 (2003)), peptide nucleic acid probes (Oliveira, K. et al., J. Clin. Microbiol. 41:889-891 (2003)), multiplex PCR (Mason, W. J. et al., J. Clin. Microbiol. 39:3332-3338 (2001)), gel-based PCR (Krishnan, P.U. et al., J. Clin Pathol. 55:745-748 (2002)), and real-time PCR (Shrestha N.K. et al., J. Clin. Microbiol. 40:2659-2661 (2002); Tan, T.Y. et al., J. Clin. Microbiol. 39:4529-4531 (2001)).

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However, the use of such molecular assays suffers from two main restrictions: First, they rely on a pre-identification of the pathogen since their discriminatory capacity is technically limited, for instance by the number of fluorochromes available for labelling the probes or, in the case of multiplex PCR, by the capacity of resolution in gel electrophoresis. These molecular assays are thus usually not scalable and unfit for high throughput analysis.

The last years have witnessed the emergence of many DNA microchip projects arraying genes of microorganisms (Ye, R.W. et al., J. Microbiol. Methods 47:257-272 (2001)). They can detect tens of thousands of DNA sequences in a single hybridisation step (DeRisi, J.L. et al., Science 278:680-686 (1997); Duggan, D.J. et al., Nat. Genet. 21:10-14 (1999); Lashkari, D.A. et al., Proc. Natl. Acad. Sci. USA

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94:13057-13062 (1997)). Originally developed for gene expression profiling, DNA sequence analysis and genotyping, microarrays were recently also used to identify viral (Wang, R.F. et al., FEMS Microbiol. Lett. 213:175-182 (2002)) and bacterial (Bekal, S. et al., J. Clin. Microbiol. 41:2113-2125 (2003)) pathogens in environmental and clinical samples.

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Most of the published reports employed oligonucleotide microarrays containing a reduced number of spotted probes and representing a single bacterial species only (Volokhov, D. et al., J. Appl. Microbiol. 95:787-798 (2003); Volokhov, D. et al., J. Clin. Microbiol. 41:4071-4080 (2003); Volokhov, D. et al., J. Clin. Microbiol. 40:4720-4728 (2002)). Such arrays were used to identify pathogenic strains belonging to a pre-identified species (Chizhikov, V. et al., Appl. Environ. Microbiol. 67:3258-3263 (2001)), to distinguish between species of the same genus (Volokhov, D. et al., J. Clin. Microbiol. 41:4071-4080 (2003); Volokhov, D. et al., J. Clin. Microbiol. 40:4720-4728 (2002)) or to detect genes encoding resistance to a certain antibiotic (Volokhov, D. et al., J. Appl. Microbiol. 95:787-798 (2003)).

Further microarrays for detection of bacteria and fungi are known in the art (Nakamura, M. et al., Abstracts of the general meeting of the American society for microbiology, abstract No C219 (2003); Wang, R.-F. et al., Molecular and Cellular Probes 223-224 (2004); Lehner, A. et al., FEMS Microbiol. Lett. 133-142 (2005); EP 1310569; WO 92/07096; US-B1-6,747,137). However, all these microarrays have in common the use of short oligonucleotides with a maximum length of 40 nt ("short oligonucleotides"). They are short-oligonucleotide microarrays. Although such short-oligonucleotide microarrays could be rapidly designed and built up they carry some intrinsic disadvantages: like all methods based on single and often short DNA sequences they show reduced reliability and sensitivity (Stears, R.L. et al., Nat. Med. 9:140-145 (2003)). To palliate the high probability of non-specific hybridisation due to the short size (20-40 bp) of the oligonucleotides it is necessary to design many partially overlapping oligonucleotides in order to confirm the presence of a gene. This consequent increase in complexity makes it extremely difficult to set up the optimal hybridisation conditions necessary for producing trustful results. Moreover, surface-bound short oligonucleotides have poor hybridisation properties and are highly sensitive to single nucleotide polymorphisms (Hughes, T.R. et al., Nat. Biotechnol. 19:342-347 (2001)). For these reasons, oligonucleotide microarrays using oligonucleotides with a maximum length of 40 nt are unsuitable for routine diagnostics.

Up to now, diagnosis of bacteremia by microarrays is limited to species identification by oligonucleotides for 23S and 18S RNA sequences, which is still strictly experimental (Anthony, R.M. et al., J. Clin. Microbiol. 38:781-788 (2000)) and carries along the methodological weakness associated to the use of short oligonucleotides as hybridisation probes.

A DNA microarray employing capture probes of more than 40 nt length amplified by PCR was described by Fitzgerald et al. (Fitzgerald, J.R. at al., Proc. Natl. Acad. Sci. USA 98(15):8821-8826 (2001)). To investigate molecular population genetics of *Staphylococcus aureus* on a genome scale, a microarray comprising 2817 complete ORFs of *S. aureus* strain COL was constructed, representing >90% of the *S. aureus* genome. The microarray was able to discriminate 36 *S. aureus* strains. However, since it was not designed for the identification of different bacterial species, it was not tested for possible cross reactions with other bacteria besides *S. aureus*. Due to the conservative nature of many house-keeping proteins and genes, respectively, cross reactions of the microarray with CoNS strains and other bacterial species will occur. Unspecific cross reactions combined with the high number of probes (2817) result in a high complexity of the microarray data, not applicable to routine diagnostics. Furthermore, PCR amplification of long ORFs is a difficult procedure, in particular for bacteria with DNA of high GC-content.

The aim of present invention is to provide a gene-segment based analytical device, especially a microarray, for species specific identification and characterisation of different microorganisms, especially different bacteria and pathogenic fungi, present in a sample or clinical specimen which does not possess the drawbacks of the short-oligonucleotide microarray as outlined above. Said device/microarray must allow the specific identification of the target species and should furthermore allow the differentiation (i.e. distinguish) between different target microorganisms present in the sample or clinical specimen. It must furthermore provide a high reliability and sensitivity of detection.

### **Summary of the Invention**

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The present invention provides an analytical device, which is preferably a DNA microarray, for the identification and characterisation of microorganisms in

biological samples, especially of microorganisms connected with bacteremia, fungemia and sepsis. Species specific gene probes in this device/microarray allow the identification of different microbial species, whilst antibiotic resistance and virulence gene probes allow for the genotypic discrimination within a species. The device/microarray can be designed to allow species identification, virulence determination and resistance determination independently from each other or simultaneously, and furthermore said determinations can be performed for one or more different microbial species and strains with one device/microarray. Furthermore, different microbial species and strains are discriminated, even in a polymicrobial sample (specimen with more than one pathogen).

The device/DNA microarray according to present invention thus demonstrates the feasibility of simultaneously identifying and characterising different microbial species in a sample or clinical specimen, especially in blood samples, without prior PCR amplification of target DNA or pre-identification of the pathogen. This can reduce sample processing time to a single day and less.

The invention furthermore provides a method for rapid identification and characterisation of microorganisms, especially of bacteria, yeasts and filamentous fungi, using the device/microarray of the invention. The method is quick, can be automated, leads to reproducible results and allows an early choice of specific antibiotics for treatment of bacteremia, fungemia or sepsis.

In particular, the present invention provides

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- (1) an analytical device for direct identification and characterisation of microorganisms in a sample or clinical specimen, wherein the analytical device comprises species specific gene probes which are (i) selected from DNA sequences or partial DNA sequences of the microorganisms to be identified or DNA sequences complementary or homologous thereto, and (ii) have a length of at least 100 nucleotides (nt);
- (2) the use of the analytical device as defined in (1) above for *in vitro* identification and characterisation of microorganisms in a sample or in a clinical specimen, preferably in a clinical specimen, more preferably for the diagnosis of a clinical condition, most preferably for the diagnosis of bacteremia, fungemia or sepsis;
- (3) an *in vitro* method for identification and characterisation of microorganisms in a sample or in a clinical specimen comprising

- (a) isolating the total DNA from the sample or clinical specimen and labelling the DNA with a reporter molecule, preferably a fluorochrome;
- (b) applying the DNA thus obtained to the analytical device as defined in (1) above and hybridising the DNA with the gene probes of the device; and
- (c) detecting DNA bound to the device by determination of the amount of the reporter molecules bound to the device; and
- (4) a kit for detection of microorganisms in a sample or clinical specimen comprising the analytical device of embodiment (1).

### **Brief description of the Figures**

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- 10 <u>Fig. 1:</u> DNA microarray analyses of 58 clinical isolates, reference strains and blood cultures.
  - Each column shows the results of an individual hybridisation with target DNA prepared from: *S. aureus* ATCC 29213 (1), MW2 (2), clinical isolates (3-7), positive blood cultures (8-11); *P. aeruginosa* ATCC 27853 (12), clinical isolates (13-17), positive blood culture (18); *E. coli* ATCC 25922 (19), clinical isolates (20-25), positive blood cultures (26-27); *S. epidermidis* clinical isolates (28-32), positive blood cultures (33-35); clinical isolates of *S. auricularis* (36), *S. capitis* (37), *S. haemolyticus* (38), *S. hominis* (39), and *S. warneri* (40). Other Gram-negative species included a *Proteus mirabilis* positive blood culture (41), clinical isolates of *Proteus mirabilis* (42-43), *Serratia marcescens* (44-45), *Klebsiella pneumonia* (46-48), *Stenotrophomonas maltophilia* (49), *Acinetobacter baumannii* (50), *Enterobacter cloacae* (51) and *Enterobacter aerogenes* (52); other Gram-positive species included clinical isolates of *Micrococcus* spp. (53), *Enterococcus* spp. (54), *Enterococcus faecalis* (55) and *Streptococcus pneumoniae* (56) and two positive blood cultures of *S. pneumoniae* (57-58).
  - (A) Hybridisation of DNA prepared from bacterial isolates, reference strains and blood cultures with *E. coli* gene probes;
  - (B) hybridisation with P. aeruginosa gene probes;
  - (C) hybridisation with S. aureus gene probes.
- 30 Grey boxes represent gene probes which hybridised with the respective target DNA, white boxes represent gene probes which showed no hybridisation with the respective target DNA.

- <u>Fig. 2:</u> Validation of the *S. aureus* microarray of example 1.11. 2  $\mu$ g genomic DNA from *S. aureus* strain T94 were labelled either with Cy3 or Cy5, combined and hybridised as described in Example 1.11. Cy3: green signal; Cy5: red signal; double-hybridisation: yellow signal.
- 5 A) Overlay of microarray scanned using Cy3 and Cy5 filter sets;
  - B) Scatterplot of normalized fluorescence intensities of individual gene probes after microarray hybridisation. The signal intensities from both channels correlate highly with each other ( $r^2 = 0.97$ ).
- Fig. 3: Specific identification of *S. aureus* from distantly related bacteria using the microarray of example 1.11. 2 μg of *S. aureus* DNA were co-hybridised with 2 μg of pure *E. coli* (A) or *P. aeruginosa* (B) genomic DNA. Obtained hybridisation patterns are represented as bar codes, where the 140 spotted gene segments appear subsequently and are clustered in categories (NC: negative control; PC: positive control; Antibiotic Resistance Determinants; Virulence Factors and Metabolic
  Functions (see Tab. 6)). Positive hybridisation is indicated by a bar while negative spots are represented by an empty area. Both assays show clear *S. aureus* discrimination with practically no cross hybridisation between DNA from said gram negative bacteria and *S. aureus* selected genes, while the positive control (16S RNA sequence) reveals the good quality of hybridisation.
- Fig. 4: Specific identification of *S. aureus* from coagulase negative staphylococci using the microarray of example 1.11. 2 μg of *S. aureus* DNA were co-hybridised with 2 μg of *S. epidermidis* (A) or *S. saprophyticus* (B) genomic DNA. Obtained hybridisation patterns are illustrated by scanned fluorescent picture data (A: *S. aureus*: green signal; *S. epidermidis*: red signal; B: *S. aureus*: red signal; *S. saprophyticus*: green signal) and transformed in bar codes (see legend of Fig. 3). All specific *S. aureus* virulence factor genes hybridised exclusively with *S. aureus* DNA. Yellow spots showing cross-hybridisation correspond to some shared antibiotic resistance determinants and genes associated to metabolic functions.
  - Fig. 5: Specificity of the S. aureus microarray of example 1.11.
- A) Scan of microarray hybridised with 2 μg each of genomic DNA from *S. aureus* strain T103 (Cy3, represented in green) or T100 (Cy5, represented in red), showing remarkable genotypic differences between strains.

- B) PCR amplification of the genes from genomic DNA of *S. aureus* (strains T100 and T103) validating results of the microarray hybridisation shown in (A).
- <u>Fig. 6:</u> Identification and characterisation of *S. aureus* from positive blood culture using the microarray of example 1.11.
- 2 μg of DNA prepared from blood culture positive for *S. aureus* (strain T95) was cohybridised with 2 μg of DNA prepared from sterile blood culture or with 2 μg of pure *S. aureus* genomic DNA for 4 hours. Positive and negative spots are transformed in a bar code scheme (see legend of Fig. 3).
- Sterile blood culture DNA did not cross-hybridise with spotted *S. aureus* genes (A).

  Blood culture positive for *S. aureus* produced a fluorescent hybridisation pattern almost identical to the pattern obtained with pure *S. aureus* genomic DNA (B).
- Fig. 7: Hybridization profiles obtained in Example 2 after microarray hybridization with DNA obtained from six bacterial target strains: (A) *S. aureus* ATCC 29213, (B) *S. epidermidis* BC 1920, (C) *S. pyogenes* DSM 11723, (D) *S. pneumoniae* ATCC 49619, (E) *E. faecalis* UW 700700/95, (F) *E. faecium* VRE9182 and two non-target strains: (G) *E. casseliflavus* UW703/95 and (H) *S. angiosus* DSM 20563.. Each bar represents the fluorescent signal of one capture probe. Fluorescent signals of the 930 probes represent the median intensity of four spots from which the local background was substracted. Probe IDs are given in Table 8.
- Fig. 8: Specificity of the microarray for *Candida albicans* in Example 2. (A) Hybridization profile obtained for *C. albicans* ATCC 10231. (B) Specificity of two *C. albicans* capture probes. Hybridization signals were determined for the two probes after hybridization with DNA obtained from 44 different microbial strains (see Table 9 for strain identification).
- 25 <u>Fig. 9:</u> Specificity of selected capture probes for (A) *Klebsiella oxytoca*, (B) *K. pneumoniae*, (C) *Proteus vulgaris* and (D) *P. mirabilis* does allow species discrimination. Fluorescence intensities refer to hybridization signals obtained for the respective probes after hybridization with DNA isolated from 44 different microbial strains (see Table 9 for strain identification).
- 30 <u>Fig. 10:</u> Specificity of selected capture probes for the coagulase-negative staphylococci (A) *S. epidermidis*, (B) *S. haemolyticus*, (C) *S. warneri* and (D) *S. saprophyticus*. Fluorescence intensities refer to hybridization signals obtained for

the respective probes after hybridization with DNA isolated from 44 different microbial strains (see Table 9 for strain identification).

#### **Definitions**

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In the framework of the present invention the following terms and definitions are used.

An "analytical device" in the context of present invention is any solid support onto which DNA gene probes are attached in a way permitting hybridisation of the DNA in the sample and subsequent detection of the bound DNA. This includes microtiter plates coated with one or several DNA gene probes per well, glass surfaces (like, e.g., microscopic slides) with DNA spots, filter paper disks, membranes, gold electrodes and beads (particles with a diameter of from 1 nm to several µm made of glass, plastic, metal etc.) coated with DNA, etc.. The beads may be used in a multi-chamber system, preferably in a microfluidic multi-chamber system, wherein each chamber contains a population of beads. Each bead has an attached DNA sequence and the whole beads population in one chamber will carry the same DNA sequence, each chamber corresponding then to a specific capture probe. The target DNA to be analysed flows through the multi-chamber system and will hybridize with the complementary DNA sequences attached to the beads. Beads could be also attached to a surface by magnetic force, i.e. paramagnetic beads coupled with DNA could be attached on the surface of the magnet and arrange in a lattice structure. Vice versa, beads made of a magnetic material could be attached to an iron surface.

The analytical device of present application is preferably a DNA microarray, a (magnetic) bead or set of beads coated with DNA probes or a microtiter plate coated with DNA probes. More preferred it is a (magnetic) bead or set of beads coated with DNA probes or a DNA microarray. In the most preferred aspect of present invention it is a DNA microarray.

A "DNA microarray" consists of a collection of nucleic acid sequences, preferably DNA sequences, immobilized onto a solid support, such as glass, plastic or silicon chips, in a latticed pattern (forming an "array"). Each unique sequence of said sequences forms a tiny feature on the microarray called a "spot" or "capture probe". The size of these spots varies from one system to another, but is usually

less than two hundred micrometers in diameter, thus up to tens of thousands of spots can be arrayed in a total area of a few square centimeters. DNA microarrays provide a means to detect and quantify large numbers of discrete nucleic sequences in parallel. In a microarray hybridisation the nucleic acids in the sample that is being analysed (called "target") are expected to form duplexes specifically with the corresponding capture probes. Occurrence or absence of duplex formation indicate the presence or absence of said target. For routine microarray analysis, said target is commonly converted to a labelled population of nucleic acids, using reporter molecules. Hybridisation of said labelled target DNA molecules from the tested samples with complementary DNA sequences affixed in specific spots on the array can thus be detected by examination for the presence of said label on the array using a microarray scanner (Müller, H.-J., Röder, T., "Der Experimentator: Microarrays", Spektrum Akademischer Verlag, Heidelberg (2004)).

In the following, the invention is exemplified for a DNA microarray (synonym: "array"). The invention can, however, also be performed using any other of the analytical devices as listed above.

"Gene probe" or "gene probe derived from..." refers to a DNA sequence present on the microarray of present invention and used as a capture probe. It is a DNA segment (see below) which is complementary to a target DNA sequence, preferably to a microbial, more preferably to a bacterial or fungal gene or gene segment. Said gene probe is prepared by any known method of DNA synthesis, and preferably prepared by cloning the respective PCR-amplified gene or gene segment into a plasmid/vector. The recombinant gene or gene segment is then amplified by PCR, isolated from the amplification mix, purified (preferably by ethanol-purification) and finally spotted onto the array.

An "isolate" is a microbial, especially a fungal or bacterial strain isolated from a given specimen, wherein the isolation includes at least one *in vitro* propagation.

A "clinical isolate" is an isolate from a clinical specimen.

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"Coagulase-negative staphylococci" ("CoNS") are bacteria of the genus Staphylococcus which are negative for a bacterial coagulase (do not induce clotting of a serum). These are all Staphylococci with the exception of *S. aureus*. Preferred CoNS in the context of present invention are *Staphylococcus epidermidis*,

Staphylococcus haemolyticus, Staphylococcus lugdunensis and Staphylococcus warneri, of which Staphylococcus epidermidis is especially preferred.

An "isolated DNA" is a DNA separated or purified from the organism it is naturally associated with or from the clinical specimen in which it occurs. This comprises biochemically or biophysically purified native DNA, recombinant DNA, chemically synthesized DNA and DNA analogues (e.g. peptide nucleic acids).

"Native" is synonymous to "naturally (occuring)".

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A "DNA segment" or "gene segment" is an isolated DNA which contains or consists of a part of the native full-length sequence of a gene which is still able to hybridize to the native sequence under stringent hybridisation conditions. Although the present invention is in the following exclusively described as relating to "DNA" sequences, it is not to be construed as being limited thereto. Rather, if the term "DNA" is used in connection with the gene probes or target sequences of present invention, it includes other polynucleotides (like RNA or RNA/DNA hybrids), and DNA analogues such as PNA, phosphonate backbone DNA, artificial pentose or hexose backbone DNA which is able to hybridize with native DNA etc.. Furthermore, modified bases like deoxy bases, inosine or aminoallylcytosine may be used on all DNA, RNA and PNA backbones. However, DNA itself is the preferred polynucleotide for performance of the invention.

The DNA sequences used as gene probes in present invention are either identical, substantially identical or homologous to the complementary native target sequences (i.e. they are "derived from" said target sequences). In the context of present invention, when a specific DNA sequence is denominated, this encompasses not only said specific sequence, but also the sequences substantially identical or homologous thereto, i.e. its substitution mutants. "Substantially identical" means that the DNA contains mutations of up to 10% of the total number of nt in comparison with the native DNA sequence and/or has a nucleotide identity of > 90% to the corresponding native DNA segment. Said mutations are preferably single nucleotide polymorphisms or point mutations and include the mutation of not only a single but also a few (up to 10 nt, preferably up to 5 nt) consecutive nt. "Homologous" or "homologue" refers to a DNA sequence which has a sequence identity of more than 70% of the corresponding native DNA sequence and encompasses the substantially identical DNA sequences. Preferably, the sequences

used as gene probes are at least substantially identical to the corresponding native DNA sequence.

Preferred gene probes of the present invention are the DNA sequences listed in the sequence protocol, their complementary sequences or their corresponding native DNA segment.

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The DNA sequences used as gene probes in present invention may also be deletion or addition mutants of the corresponding native DNA segments. In case of deletion mutants, the minimum length of the DNA sequences suitable as probes in present invention is 100 nt. Preferably, the deletions take place at the 5'- and/or 3'terminus of the native DNA segment. In case of addition mutants, the added nucleotides may sum up to a total of 90% of the nucleotide number of the native DNA segment, if added at the 5'- or 3'-terminus of the DNA sequence. Alternatively, the additions and deletions may be of one isolated nucleotide or of 2 or more consecutive nucleotides at one or more internal site(s) of the native DNA segment. Preferably, 0-30% nucleotides of the corresponding native DNA segment are added or deleted. It is most preferred that the addition or deletion mutants used as gene probes in present invention comprise one or more segment(s) of at least 100 consecutive nt each, which are derived from one gene, and/or sequences homologous (70% homology) or complementary thereto. These segments may be embedded in or fused to other DNA sequences, which will not hybridize under stringent conditions with either human or bacterial DNA or the DNA of the target microorganism. Said other DNA sequences preferably have a maximum length which adds up with the length of the enclosed segment(s) to not more than the upper limit for the length of gene probes suitable for present invention.

A "positive blood culture" is an *in vitro* culture started from whole blood or blood components wherein the growth of microorganisms has been detected. Said growth is indicated by a positive growth index. The detection is preferably done by monitoring CO<sub>2</sub> production in the blood culture.

"Direct identification" of microorganisms refers to an identification method which comprises isolation of DNA from a sample or clinical specimen, but does not require an amplification of the genetic material of the microorganisms after said isolation in order to identify the microorganisms using the method of present invention. The isolated genetic material is labelled and applied to the DNA microarray of present

invention without prior amplification, i.e. directly after isolation or after a short workup step.

"Species-specific" probe(s) means that a species can be identified specifically and unambiguously using said probe or set of probes.

5 "Differentiation" means the discrimination among distinct and different species, genera or groups of pathogens.

A "detection method" in the context of the present invention is a method for determination of hybridisation of DNA molecules contained in a sample to the probes on the solid support of the microarray of present invention. This method may be any textbook method for detection of DNA hybridisation on microarrays, e.g. direct detection or labelling of target DNA with a reporter molecule and consecutive visualisation of the reporter molecule. Preferred detection methods are said labelling method and the direct detection by electrical biosensors or mass spectrometry (Liu, R. H. et al., Anal. Chem. 76(7):1824-31 (2004); Stomakhin, A. A. et al., Nucleic Acids Res. 28(5):1193-8 (2000)).

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A "reporter molecule" in the context of the method of the present invention is a chemical or physical marker which allows differentiation of labelled from unlabelled DNA by physical, chemical or immunological methods. The labelling method includes, but is not limited to radioactive labelling (e.g. with 33P, 32P), fluorescent/luminescent/chromophor labelling and hapten labelling (i.e. psoralen or DIG). It is followed by an appropriate detection step necessary to determine the presence and/or quantity of the reporter molecule, namely scintillation counting (e.g. phosphoimaging); photooptic measurement (e.g. fluorescence measurement, luminescence measurement) and antibody-based detection (including colorimetric, luminescence or fluorescence detection), respectively. Preferably, the reporter molecule is a fluorochrome/fluorophor (both terms are used as synonyms in the context of present invention) which includes but is not limited to cyanines, fluoresceins and rhodamines. More preferably, it is of the cyanine group of fluorophores. Most preferably, it is selected from the group consisting of the fluorophores Cy3, Cy5 or Alexa Fluor 647 and Alexa Fluor 546. The ratio of base to dye molecules (BDR) in DNA labelled with such reporter molecules is preferably less or equal to 60.

A "target species" is a species for which species-specific capture probes are present in the microarray, allowing species identification by positive hybridisation. "Nontarget species" are all other species.

# **Detailed description of the invention**

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The present invention provides an analytical device, preferably a DNA microarray, and its use for rapid identification and characterisation of microorganisms in a sample or clinical specimen (embodiments (1) to (3)). The invention is exemplified in the following by the most preferred embodiment of the analytical device (1), namely a DNA microarray. The invention can, however, also be performed using any other of the analytical devices as listed above. Thus, unless otherwise stated, in the following the term "DNA microarray of embodiment (1)" is to be understood as "analytical device of embodiment (1)".

The DNA microarray of embodiment (1) of the invention comprises gene specific DNA sequences as capture probes, which allow the identification of microbial species ("target species"), especially of bacterial and fungal species, and/or their further characterisation with regard to antibiotic resistance and virulence. Preferably, it allows the identification and characterisation of the target species. It is specific, applicable to the analysis of DNA isolated from blood cultures and suitable to detect resistance genes.

The DNA microarray of embodiment (1) comprises at least 1 species specific probe per target species. In a preferred aspect of the invention, it additionally comprises one or more virulence and/or resistance gene probe(s).

A further preferred aspect of embodiment (1) is that the DNA microarray comprises species specific probes for more than one or multiple microbial species, i.e. for a plurality of species. The DNA microarray of this preferred aspect of embodiment (1) allows the simultaneous detection of a plurality of microbial species in a sample without previous isolation and/or amplification of single species. It furthermore allows a one-step determination of whether certain microorganisms are present in a sample or not, even if the sample comprises a plurality of different microbial strains.

One important feature of the microarray of the present invention is that the panel of probes can be continually extended to include sequences for additional species, variant isolates or antibiotic resistance determinants as they are characterised and available. The accuracy, range and discriminatory power of the gene-segment based microarray can be refined by adding or removing gene probes to the panel without significantly increasing complexity or costs. In a pilot study, three important species causing bacteremia were selected to provide a proof of principle (examples 1.1-1.10). The range of organisms that can be identified can be easily expanded by increasing the number of gene probes on the array. For example, addition of a few probes specific for *S. epidermidis* and other CoNS will allow for the species identification of coagulase-negative staphylococci. Furthermore, due to a specific hybridisation pattern for each species it will also allow the identification of mixed blood cultures with more than one pathogen.

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A second important feature of this microarray format is the length of the DNA sequences used as gene probes. They are at least 100 nt, preferably 100-3000 nt long. In an especially preferred aspect of embodiment (1) the length of the gene probes is from 100 to 1000 nt, most preferably from 200 to 800 nt. Thus, one probe per gene is usually sufficient to produce strong signals and high specificity (Stears, R.L. et al., Nat. Med., 9:140-5 (2003)). For long probes like these, minor point mutations are likely to only slightly reduce duplex formation, which does not lead to the loss of hybridisation signals. In contrast, short oligonucleotide microarrays sometimes lack specificity and require multiple short oligonucleotides per one gene.

The microorganisms or microbial DNA to be detected using the microarray of present invention are preferably bacteria (such as *Staphylococci, Enterococci, Streptococci, E. coli, P. aeruginosa, Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Acinetobacter* spp. and *Stenotrophomonas* spp.) or fungi (such as yeasts and filamentous fungi, in particular *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp., *Malassezia* spp., *Trichosporin* spp.), respectively bacterial or fungal DNA. The microarray is especially suitable for direct identification and characterisation of bacteria and *C. albicans*.

In a preferred aspect of embodiment (1) the analytical device is suitable for species specific identification of one microbial strain or (preferably) a plurality of microbial strains in clinical specimens comprising microbial strains, especially bacteria and/or fungi. It furthermore allows differentiation of the target species from each other

and from non-target-species contained in one sample comprising a plurality of microbial strains.

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In one preferred aspect of embodiments (1), (2) and (3), the DNA microarray is feasible to identify and characterize any of the microorganisms, including the fungi and bacteria as defined above, known as etiological agents of fungemia, bacteremia or sepsis. In another preferred aspect of (1), it is feasible to characterize the bacteria known as etiological agents of bacteremia or sepsis. More preferably, it is feasible to identify and characterize at least 90 % of said microorganisms or bacteria. Equally more preferably it is feasible to identify and characterize microorganisms selected from the group consisting of S. aureus, Coagulasenegative staphylococci, Enterococci, Streptococci, E. coli, Klebsiella spp., Proteus spp, P. aeruginosa, Acinetobacter spp. and Candida albicans, most preferably microorganisms selected from the group consisting of S. aureus, CoNS (including Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus warneri, Staphylococcus saprophyticus, Staphylococcus hominis), C. albicans, Enterococcus faecalis, Enterococcus faecium, E. coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, P. aeruginosa, Acinetobacter baumannii, Streptococcus agalactiae, Streptococcus bovis, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes.

In a first most preferred aspect of embodiment (1), the DNA microarray is suitable for species specific identification of microorganisms selected from the group consisting of Staphylococci, *E. coli* and Candida sp., preferably for species specific identification of Staphylococci, especially of *S. aureus*. More preferably, it is suitable for species specific identification of Staphylococci and at least one of *E. coli* and *Candida albicans*.

In a second most preferred aspect of embodiment (1), the DNA microarray is suitable to identify and characterize at least *S. aureus, Coagulase-negative staphylococci, E. coli, Enterococcus faecalis* and *faecium* and *Candida albicans*.

In addition to above aspects, the DNA microarray is in a preferred embodiment of present invention suitable for additional species specific identification or differentiation of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Streptococcus* 

pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus mirabilis and/or Proteus vulgaris.

The practicability and specificity of the DNA microarray for the identification and characterisation of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was evaluated with clinical isolates and positive blood cultures (Examples 1.1-1.10). Especially preferred is a microarray which allows identification and characterisation of *S. aureus*. The latter microarray allows the detection of every *S. aureus* isolate, unambiguously identifies most of important virulence genes such as *tsst-1*, *sea*, *seb*, *eta* and antibiotic resistance genes such as *mecA*, *aacA-aphD*, *blaZ*, *ermA* and specifically distinguishes *S. aureus* from unrelated gram negative bacteria, e.g. *Escherichia coli* or *Pseudomonas aeruginosa*, as well as from closely related CoNS (Example 1.11, Fig. 2-6).

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In another preferred aspect of the invention, the microarray of (1) is suitable for diagnosis of fungemia, bacteremia or sepsis; especially for diagnosis of bacteremia, candidemia, and bacterial or *Candida* sepsis.

The present invention provides a novel approach for detection of microorganisms, especially of bacteria and fungi, by microarrays: using gene-segments it allows species identification by probing a large and diverse set of species-specific genes. Such an approach is reliable since it makes possible to identify a pathogen even when some genes have been deleted from its genome. Furthermore, the selected DNA probes are at least 100 nt, preferably 200 to 800 nt long and are therefore not sensitive to single nucleotide polymorphisms or CG-content variations in the targets. Therefore, a gene segment array according to present invention is useful for indicating the presence of a gene even though the sequence may be slightly altered e.g. by point mutations (Southern, E. et al., Nat. Genet. 21:5-9 (1999)). Additionally, it permits species virulence and antibiotics resistance profiling all together in a single-step test. Thus, present invention provides for a significant improvement compared to the classical approach focused on the detection of a short evolutionary conserved sequence like 16S RNA.

The number and perfect composition of gene-segments necessary for a correct species identification, virulence determination and resistance profiling must be determined by empiric specificity tests. Thus, in a preferred aspect of the invention, the DNA microarray of embodiment (1) comprises the minimal number of species

specific gene probes which is sufficient for species identification, the minimal number of virulence gene probes which is sufficient for virulence determination, and/or the minimal number of resistance gene probes which is sufficient for determination of resistance of a specific microorganism. Preferably, the minimal number of gene probes in this aspect of the invention is: for correct species identification at least 1 species specific gene probes per target species, more preferably at least 2 different species specific gene probes per target species, even more preferably at least 10, most preferably at least 20; for virulence determination at least 1 gene probe per target species, more preferably at least 5 different gene probes, even more preferably at least 20 different gene probes, most preferably gene probes for all known virulence factors of each target species; for determination of resistance at least 1 gene probe per antibiotic class or resistance factor, more preferably at least 5 different gene probes, most preferably all known gene-coded resistance determinants in the target species.

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- Generally, the DNA microarray of embodiment (1) comprises gene probes which are specific for a microbial species, bacterial/fungal species or a group of microorganisms to be identified. Said gene probes are preferably DNA sequences selected from three different groups, namely (a) species specific gene probes; (b) virulence gene probes; and/or (c) resistance gene probes.
- 20 Preferably, the species specific set of gene probes for each species to be identified and characterised is selected from species specific gene probes (a) for
  - (i) Staphylococcus aureus including gene probes derived from clfA, clfB, coa, lytM, NAG, sodA, sodB, epiP-bsaP, geh, hemC, hemD, hsdS, lip, menC, nuc, SAV0431, SAV0440, SAV0441, spa, ebpS, fbpA, fib, fnbB, srtA, stpC, fnbA, femA, fmhB, fmhA;
  - (ii) Escherichia coli including gene probes derived from b1169, fliCb, nfrB, yacH, ycdS, yciQ, shuA;
  - (iii) Staphylococcus epidermidis including gene probes derived from ardeSE0106, ardeSE0107, atlE, agrB, alphSE1368, gad, glucSE1191, icaB, mvaSSepid, nitreSE1972, nitreSE1974, nitreSE1975, oiamtSE1209, ORF1Sepid, ORF3bSepid, qacR, ureSE1865, ureSE1867;
  - (iv) Staphylococcus haemolyticus including gene probes derived from femBShaemolyt, mvaDShaemolyt, mvaSShaemolyticus, RNApolsigm;

- (v) Staphylococcus lugdunensis including gene probes derived from agrB2Stalugd, agrC2Stalugd, slamStalugd;
- (vi) Staphylococcus warneri including gene probes derived from msrw1Stwar, nukMStwar, proDStwar, proMStwar, sigrpoStwar, tnpStwar;
- 5 (vii) Staphylococcus saprophyticus including gene probes derived from RNApolsigmSsapro;
  - (viii) Staphylococcus hominis including gene probes derived from ydhK;

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- (ix) Candida albicans including gene probes derived from ARG56, ASL43f, BGL2, CCT8, CDC37, CEF3, CHS1, CHS2, CHS4, CHS5, CHT1, CHT2, CHT4, CSA1,
- 5triphosphatase, AAF1, ADH1, ALS1, ALS7, EDT1, ELF, ESS1, FAL1, GAP1, GNA1, GSC1, GSL1, HIS1, HTS1, HWP1, HYR1, INT1a, KRE15f, KRE6, KRE9, MIG1, MLS1, MP65, NDE1, PFK2, PHR1, PHR2, PHR3, PRA1, PRS1, RBT1, RBT4, RHO1, RNR1, RPB7, RPL13, RVS167, SHA3, SKN1, SRB1, TCA1, TRP1, YAE1, YRB1, YST1exon2;
  - (x) Enterococcus faecalis including gene probes derived from arcA, arcC, bkdA, camE1, csrA, dacA, dfr, dhoD1a, ABC-eltA, agrBfs, agrCfs, dnaE, ebsA, ebsB, eep, efaR, gls24\_glsB, gph, gyrAEf, metEf, mntHCb2, mob2, mvaD, mvaE, parC, pcfG, phoZ, polC, ptb, recS1, rpoN, tms, tyrDC, tyrS;
    - (xi) Enterococcus faecium including gene probes derived from bglB, bglR, bglS, efmA, efmB, efmC, mreC, mreD, mvaDEfaecium, mvaEEfaecium, mvaK1Efaecium, mvaK2Efaecium, mvaSEfaecium, orf3\_4Efaeciumb, orf6\_7Efaecium, orf7\_8Efaecium, orf9\_10Efaecium;
    - (xii) Klebsiella pneumonia including gene probes derived from atsA, budC, citA, citW, citX, dalK, acoA, acoB, acoC, ahlK, fimK, glfKPN2, ltrA, mdcC, mdcH, nifF, nifK, nifN, tyrP, wbbO, wzb, wzmKPN2, wztKPN2, yojH, liac;
- 25 (xiii) *Klebsiella oxytoca* including gene probes derived from *gatY*, *pelX*, *tagH*, *tagT*;
  - (xiv) *Pseudomonas aeruginosa* including gene probes derived from *glpR*, *lasRb*, *OrfX*, *pa0260*, *pa0572*, *pa0625*, *pa0636*, *pa1046*, *pa1069*, *pa1846*, *pa3866*, *pa4082*, *pilAp*, *PilAp2*, *pilC*, *PstP*, *uvrDII*, *vsmI*, *vsmR*, *xcpX*;
- 30 (xv) Streptococcus pneumoniae including gene probes derived from cap1EStrpneu, cap1FStrpneu, cap1GStrpneu, cap3AStrpneu, cap3BStrpneu, celAStrpneu, celBStrpneu, cglAStrpneu, cglBStrpneu, cglCStrpneu, cglDStrpneu, cinA, cps14EStrpneum, cps14FStrpneum, cps14GStrpneum, cps14HStrpneum, cps19aHStrpneum, cps19aIStrpneum, cps19aKStrpneum, cps19fGStrpneum,

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cps23fGStrpneum, dexB, dinF, 1760Strpneu, acyPStrpneu, endAStrpneu, exoAStrpneu, exp72, fnlAStrpneu, fnlBStrpneu, fnlCStrpneu, gct18Strpneum, immunofrag1Strpneu, hexB1, hftsHstrpneu, immunofrag2Strpneu, immunofrag3Strpneu, kdtBStrpneu, lysAStrpneu, pcpBStrpneu, pflCStrpneu, plpA, purRStrpneu, pyrDAStrpneum, 5 prtA1Strpneu, pspC1Strpneu, pspC2, SP0837\_38Strpneu, SP0828Strpneu, SP0830Strpneu, SP0833Strpneu, SP0839Strpneu, ugdStrpneu, uncC, vicXStrepneu, wchA6bStrpneum, wci4Strpneum, wciK4Strpneum, wciL4Strpneum, wciN6bStrpneum, wciP6bStrpneum, wciY18Strpneum, wzdbStrpneum, wciO6bStrpneum, wze6bStrpneum, wzy18Strpneum, wzy4Strpneum, wzy6bStrpneum, xpt; 10

- (xvi) Streptococcus agalactiae including gene probes derived from cpsA1Strgal, cpsB1Strgal, cpsC1Strgal, cpsD1Strgal, cpsE1Strgal, cpsG1Strgal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cpsIStragal, cylEStragal, cylFStraga, cylHStraga, cylIStraga, cylIStraga, cylIStraga, cylIStraga, cylIStraga, 0487Straga, 0488Straga, 0493Straga, 0495Straga, 0498Straga, 0500Straga, 0502Straga, 0504Straga, folDStraga, neuA1Strgal, neuB1Strgal, neuC1Strgal, neuD1Strgal, recNStraga, ileSStraga;
- (xvii) Streptococcus pyogenes including gene probes derived from cyclStrpyog, fah\_rph\_hlo\_Strpyog, int, int315.5, oppD, SPy0382Strpyog, SPy0390Strpyog, SpyM3 1351, vicXStrpyog;
- (xviii) Streptococcus mutans including gene probes derived from 573Stprmut, 580SStprmut, 581\_582SStprmut, 584SStprmut, dltAStrmut, dltBStrmut, dltCppx1Strmut, dltDStrmut, lichStrbov, lytRStprmut, lytSStprmut, pepQStrrmut, pflCStrmut, recNStprmut, ytqBStrmut;
- 25 (xix) Proteus mirabilis including gene probes derived from atfA, atfB, atfC, ccmPrmi1, cyaPrmi, flfB, flfD, flfN, flhD, floA, ftsK, gstB, hemCPrmi, hemDPrmi, hev, katA, lpp1, menE, mfd, nrpA, nrpB, nrpG, nrpS, nrpT, nrpU, pat, pmfA, pmfC, pmfE, ppaA, rsbA, rsbC, speB, stmA, stmB, terA, terD, umoA, umoB, umoC, ureR, xerC, ygbA;
- 30 (xx) *Proteus vulgaris* including gene probes derived from *envZPrvu*, *frdC*, *frdD*, *lad*, *tna2*:
  - (xxi) Acinetobacter baumanii including gene probes derived from carO, gacS, dhbA, dhbB, sid, csuD, csuC, tnp-ACIBA, waaA-ACIBA, csuB, csuA\_B, csuA, put1, por, abc, furACIBA, dec, cysI, trpE, put3, ompA-ACIBA.

Preferably, the virulence specific set of gene probes for each species to be identified and characterised is selected from virulence gene probes (b) for

- (i) Staphylococcus aureus including gene probes derived from bsaE, bsaG, cap5h, cap5i, cap5j, cap5k, cap8H, cap8I, cap8J, cap8K, I-hld, I-hysA, I-IgGbg, EDIN, eta, etb, hglA, hglB, hglC, hla, hlb, lukF, lukS, NAG, sak, sea, seb, sec1, seg, seh, sel, set15, set6, set7, set8, sprV8, tst, I-sdrC, I-sdrD, I-sdrE;
- (ii) Escherichia coli including gene probes derived from b1202, eae, eltB, escR, escT, escU, espB, fes, fteA, hlyA, hlyB, iucA, iucB, iucC, papG, rfbE, shuA, SLTII, toxA-LTPA, VT2vaB;
- 10 (iii) Staphylococcus epidermidis including gene probes derived from gcaD, hld\_orf5, icaC, icaD, icaR, psm\_beta1and2, purR, spoVG, yabJ;
  - (iv) Staphylococcus haemolyticus including gene probes derived from lipShaemolyt;
  - (v) Staphylococcus lugdunensis including gene probes derived from fblStalugd, slushABCStalugd;
- 15 (vi) Staphylococcus warneri including gene probes derived from gehAStwar;

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- (vii) Candida albicans including gene probes derived from CCN1, CDC28, CLN2, CPH1, CYB1, EFG1, MNT1, RBF1, RBF1, RIM101, RIM8, SEC14, SEC4, TUP1, YPT1, ZNF1CZF1;
- (viii) Enterococcus faecalis including gene probes derived from asa1, asp1, cgh,
   cylA, cylB, cylI, cylL\_cylS, cylM, ace, ef00108, ef00109, ef0011, ef00113, ef0012,
   ef0022, ef0031, ef0032, ef0040, ef0058, enlA, esa, esp, gelE, groEL, groES, rt1,
   sala, salb, sea1, sep1, vicK, yycH, yycI, yycJ;
  - (ix) Enterococcus faecium including gene probes derived from entA\_entI, entD, entR, oep, saqA;
- 25 (x) Klebsiella pneumonia including gene probes derived from cim, aldA, hemly, pSL017, pSL020, rcsA, rmlC, rmlD, waaG, wbbD, wbbM, wbbN, wbdA, wbdC, wztKpn, yibD;
  - (xi) *P. aeruginosa* including gene probes derived from *aprA*, *aprE*, *ctx*, *algB*, *algN*, *algR*, *ExoS*, *fpvA*, *lasRa*, *lipA*, *lipH*, *Orf159*, *Orf252*, *pchG*, *PhzA*, *PhzB*, *PLC*, *plcN*, *plcR*, *pvdD*, *pvdF*, *pyocinS1*, *pyocinS1im*, *pyocinS2*, *pys2*, *rbf303*, *rhlA*, *rhlB*, *rhlR*, *TnAP41*, *toxA*;
  - (xii) Streptococcus pneumoniae including gene probes derived from igaStrpneu, lytA, nanA, nanBStrpneu, pcpCStrpneu, ply, prtAStrpneu, pspA, SP0834Strpneu, sphtraStrpneu, wciJStrpneu, wziyStrpneu, wzxStrpneu;

- (xiii) Streptococcus agalactiae including gene probes derived from CAMPfactor, 0499Straga, hylStragal, lipStragal;
- (xiv)Streptococcus pyogenes including gene probes derived from DNaseIStrpyog, fba2Strpyog, fhuAStrpyog, fhuB1Strpyog, fhuDStrpyog, fhuGStrpyog, hylA, hylP, hylp2, oppB, ropB, scpAStrpyog, sloStrpyog, smez- Strpyog, sof, speA, speB2Strpyog, speCStrpyog, speJStrpyog, srtBStrpyog, srtCStrpyog, srtEStrpyog, srtFStrpyog, srtGStrpyog, srtTStrpyog, vicKStrpyog;

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- (xv) Streptococcus mutans including gene probes derived from hlyXStrmut, perMStrmut;
- (xvi) *Proteus mirabilis* including gene probes derived from *flaA*, *laD*, *fliA*, *hpmA*, *hpmB*, *lpsPrmi*, *mrpA*, *mrpB*, *mrpC*, *mrpD*, *mrpE*, *mrpF*, *mrpG*, *mrpH*, *mrpI*, *mrpJ*, *patA*, *putA*, *uca*, *ureDPrmi*, *ureEPrmi*, *ureFPrmi*, *zapA*, *zapB*, *zapD*, *zapE*.

Preferably, the resistance specific set of gene probes is selected from resistance gene probes (c) derived from genes coding for

- (i) beta-lactams resistance including gene probes derived from blaIMP-7, mecISepid, blaOXA-10, blaB, ampC, I-blaR, blaOXA-32, bla-CTX-M-22, pbp2aStrpneu, blaSHV-1, blaOXA-2, blaRShaemolyt, blaIMP-7, I-mecR, blaOXY, dacCStrpyog, mecA, blaIShaemolyt, blavim, pbp2b, pbp2primeSepid, pbp2x, pbp3Saureuc, pbp4, pbp5Efaecium, pbpC, I-mecI, pbp1a, I-blaI, blaTEM-106, blaOXY-KLOX, ftsWEF, cumA, blaPER-1, bla\_FOX-3, blaA, psrb, mecR1Sepid, blaZ, blaOXA-1, fox-6, blaPrmi;
- (ii) aminoglycosides resistance including gene probes derived from aacA\_aphDStwar, aacC1, aacC2, strB, aadA, aadB, aadD, aacA4, strA, aph-A3, aacC1, aacA4, aacA-aphD, I-spc, aphA3; aacA4ENCL, aac(6p)-lb7;
- (iii) macrolides-lincosamines-streptogramins resistance including gene probes derived from ermC, linB, satSA, mdrSA, I-linA, ermB, ermA, satA, msrA, mphBM, mefA, mrx;
- (iv) trimethoprim resistance including gene probes derived from dfrA, dfrStrpneu;
- 30 (v) chloramphenicol resistance including gene probes derived from *cat*, *catEfaecium*, *cmlA5*;
  - (vi) tetracyclines resistance including gene probes derived from tetAJ, tetL, tetM;

- (vii) glycopeptides resistance including gene probes derived from vanH(tn), vanA, vanHB2, vanR, vanRB2, vanS(tn), vanSB2, vanWB2, ddl, ble, vanXB2, vanY(tn), vanYB2, vanB, vanZ(tn), vanC-2, vanX(tn);
- (viii) multiple target resistance including gene probes derived from acrB, mexB, I-qacA, sulI, sul, cadBStalugd, mexA, acrR, emeA, acrA, rtn, abcXStrpmut, qacEdelta1, elkT-abcA, I-cadA, albA, wzm, msrCb, nov, wzt, wbbl, norA23, mexR, arr2, mreA, I-cadC, uvrA, AdeR-ACIBA, adeA-ACIBA, adeB-ACIBA, adeC-ACIBA, AdeS-ACIBA;

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- (ix) fungicides resistance, especially *C. albicans* fungicide resistance, including gene probes derived from *CRD2*, *CDR1*, *MET3*, *FET3*, *FTR2*, *MDR1-7*, *ERG11*, *SEC20*.
  - Most preferably, the resistance specific set of gene probes is selected from resistance gene probes (c) derived from genes coding for
  - (i) beta-lactams resistance including gene probes derived from *bla-CTX-M-22*, *blaSHV-1*, *blaTEM-106*, *mecA*, *blaZ*;
- (ii) aminoglycosides resistance including gene probes derived from aacC1, aacC2, aadA, aadB, aadD, aacA4, aph-A3, aacC1, aacA4, aacA-aphD, aphA3;
  - (iii) macrolides-lincosamines-streptogramins resistance including gene probes derived from *ermA*, *ermB*, *ermC*;
  - (iv) tetracyclines resistance including gene probes derived from tetAJ, tetL, tetM
- 20 (vii) glycopeptides resistance including gene probes derived from *vanA*, *vanB*, *vanC-2*.
  - The most relevant resistance gene probes are probes derived from and specific for *mecA*. This is due to the fact that *mecA* is common to all Staphylococci including *S. aureus* and CoNS.
- 25 Since the same resistance phenotype is determined by many different genotypes, it is preferred to use a plurality of resistance gene probes for unambiguous and comprehensive prediction of antibiotic resistance. The largest available set of resistance probes is most preferred.
- For the virulence assessment of a certain strain and the sub-species strain discrimination, it is preferred to use a plurality of virulence gene probes for unambigous and comprenehsinve virulence determination. The use of the highest available number of genotypic markers is most favourable.

Furthermore, the microarray may contain a set of gene probes which serve as controls. Preferably, such a set of control gene probes is selected from group (d) consisting of control gene probes coding for

- (i) negative controls, namely DNA sequences which will not hybridise with human DNA or bacterial, fungal or the microbial target DNA under the hybridisation conditions of the method of present invention, including gene probes derived neither from fungal, bacterial or target microbial nor from human genes, preferably gene probes derived from plant genes, more preferably from *Arabidopsis thaliana* or *Glycine max* genes;
- 10 (ii) positive controls including segments of ribosomal DNA from bacterial target species, preferably 16S DNA, and segments of conserved human genes;
  - (iii) positive controls specific for DNA added to the sample ("spiked DNA"), namely DNA sequences which will not hybridise with human DNA or the fungal, bacterial or microbial target DNA under the hybridisation conditions of the method of present invention, including gene probes derived neither from fungal, bacterial or target microbial nor from human genes, preferably gene probes derived from mouse or amoeba genes, most preferably from *Mus musculus* or *Dictyostelium discoideum* genes.

These control gene probes are necessary to

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- a) detect non-specific hybridisation;
- b) optimise hybridisation conditions and image acquisition and analysis;
- c) provide positive controls for the quality of probe preparation, hybridisation and detection; and/or
- d) control technical aspects of the entire detection procedure including labelling, hybridisation and detection steps.

In a preferred aspect of embodiment (1), the microarray contains DNA sequences selected from the group consisting of the SEQ ID NOs: 1-918 and 2842-2908, complementary sequences thereto, addition mutants, deletion mutants, substitution mutants and homologues thereof as gene probes.

More preferably, in order to identify a specific microbial species, bacterial species or group of bacteria, the gene probes of group (a) are selected from SEQ ID NO:1-99, 142-152, 174-199, 209-214, 216-219, 222-229, 231-291, 308-342, 377-393, 399-431, 449-490, 523-591, 606-639, 645-656, 687-701, 706-749, 776-781, 2843-

2863, 2902 and 2903 (compare Tab. 1). Equally, in order to determine virulence of a specific micororganism or bacterial species, the gene probes of group (b) are selected from SEQ ID NO: 100-141, 153-173, 200-208, 215, 220-221, 230, 292-307, 343-376, 394-398, 432-448, 491-522, 592-605, 640-644, 657-686, 702-705, 750-775 and 782-784 (compare Tab. 1). Equally, in order to determine antibiotic resistance of a specific microbial or bacterial species, the gene probes of group (c) are selected from SEQ ID NO:785-918, 2864-2875, 2888 and 2907-2908, preferably from SEQ ID NO:785-909, 2864-2875, 2888 and 2907-2908 (compare Tab. 1). Equally, in order to provide the required controls (negative, positive, hybridisation controls), the gene probes of group (d) are selected from SEQ ID NO:919-947, preferably from SEQ ID NO:919-925 and 944-947, more preferably from SEQ ID NO: 919 and 921 (compare Tab. 1).

<u>Tab. 1:</u> Preferred gene probes for species identification, virulence determination and resistance determination of microorganisms

### 15 a) probes for species identification

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SEQ ID NO	Probe
1	cataSaur_1_1
2	cataSaur_1_2
3	clfA_1_1
4	clfB_1_1
5	coa_1_1
6	coa_1_2
7	I-clpC_1_1
8	I-clpP_1_1
9	I-ctaA_1_1
10	I-ctsR_1_1
11	I-dltA_1_1
12	I-dltB_1_1
13	I-dltC_1_1
14	I-dnaK_1_1
15	I-elkT_1_1
16	I-femD_1_1
17	I-glnA_1_1
18	I-glnR_1_1
19	I-grlA_1_1
20	I-grlB_1_1
21	I-groEL_1_1
22	I-groES_1_1
23	I-hemA_1_1
24	I-hemE_1_1
25	I-hemH_1_1
26	I-hemL_1_1
27	I-hemY_1_1
28	I-lepA_1_1

1-lrgA 1 1   1   1   1   1   1   1   1   1	SEQ ID NO	Probe
30   I-irgB 1 1   1   1   1   1   1   1   1   1	29	I-lrgA_1_1
31	30	
32		I-lytM_1_1
34		
34		
35		I-menE 1_1
36   I-mreR 1 1   1   1   1   1   1   1   1   1		
37		
38		
1-mutS 1 1   1   1   1   1   1   1   1   1		
40		
1-pbg 1		
1-pbpF 1		
1-pdhC_1_1		I-pbpF 1 1
45		I-pdhB 1 1
45		I-pdhC 1 1
46		
47   I-rsbW_1 1   1   48   I-sgp_1 1   1   1   1   1   1   1   1   1   1		
48		
49       I-sirR 1_1         50       I-sodA 1_1         51       I-sodB_1_1         52       I-sstB_1_1         53       I-sstC_1_1         55       I-sstC_1_1         55       I-sstC_1_1         56       I-trx_1_1         57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1         66       hsdS_2_1         68       lip_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rp8_1_1         74       SAV0431_1         75       SAV0449_1_1         76       SAV0440_1_1         77       SAV0441_1_1         79       sp8_1_2         80       sstC_1_1		
50         I-sodA_1_1           51         I-sodB_1_1           52         I-sstA_1=1           53         I-sstC_1=1           54         I-sstC_1=1           55         I-sstD_1=1           56         I-trx_1=1           57         I-yhiN_1=1           58         epiP-bsaP_1=1           59         geh_1=1           60         gyrA_1=1           61         gyrB_1=1           62         hemB_1=1           63         hemC_1=1           64         hemD_1=1           65         hemN_1=1           66         hsdS_1=1           67         hsdS_2=1           68         lip_1=1           70         murC_1=1           71         nuc_1=1           72         pdhD_1=1           73         rpoB_1=1           74         SAV043=1=1           75         SAV0440=1=1           77         SAV0441=1           79         spa_1=2           80         sstC_1=1		
51       I-sodB_1_1         52       I-sstA_1_1         53       I-sstB_1         54       I-sstC_1_1         55       I-sstD_1_1         56       I-trx_1_1         57       I-yhlN_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       heMN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV043_1_1_1         75       SAV043_9_1_1         76       SAV044_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
52       I-sstA_1_1         53       I-sstB_1         54       I-sstC_1         55       I-stD_1         56       I-trx_1_1         57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1         62       hemB_1_1         63       hemC_1.1         64       hemD_1_1         65       hemN_1.1         66       hsdS_1_1         67       hsdS_2.1         68       lip_1_1         69       menC_1.1         70       murC_1_1         71       nuc_1.1         72       pdhD_1_1         73       rpoB_1.1         74       SAV0439_1.1         75       SAV0440_1.1         77       SAV0441_1.1         78       sigB_1.1         79       spa_1.2         80       sstC_1_1		
53       I-sstB_1_1         54       I-sstC_1_1         55       I-sstD_1_1         56       I-trx_1_1         57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0439_1_1         75       SAV0440_1_1         76       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
54       I-sstC_1_1         55       I-sstD_1_1         56       I-trx_1_1         57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
55         I-sstD_1_1           56         I-trx_1 1           57         I-yhiN_1_1           58         epiP-bsaP_1_1           59         geh_1_1           60         gyrA_1_1           61         gyrB_1_1           62         hemB_1_1           63         hemC_1_1           64         hemD_1_1           65         hemN_1_1           66         hsdS_1_1           67         hsdS_2_1           68         lip_1_1           69         menC_1_1           70         murC_1_1           71         nuc_1_1           72         pdhD_1_1           73         rpoB_1_1           74         SAV0431_1_1           75         SAV0439_1_1           76         SAV0440_1_1           77         SAV0441_1_1           78         sigB_1_1           79         spa_1_2           80         sstC_1_1		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
56       I-trx_1_1         57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rp0B_1_1         74       SAV0431_1_1         75       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
57       I-yhiN_1_1         58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0440_1         77       SAV0441_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
58       epiP-bsaP_1_1         59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0440_1_1         76       SAV0441_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
59       geh_1_1         60       gyrA_1_1         61       gyrB_1_1         62       hemB_1_1         63       hemC_1_1         64       hemD_1_1         65       hemN_1_1         66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
60		
61 gyrB_1_1 62 hemB_1_1 63 hemC_1_1 64 hemD_1_1 65 hemN_1_1 66 hsdS_1_1 67 hsdS_2_1 68 lip_1_1 69 menC_1_1 70 murC_1_1 71 nuc_1_1 72 pdhD_1_1 73 rpoB_1_1 74 SAV0431_1_1 75 SAV0440_1_1 76 SAV0440_1_1 77 SAV0441_1_1 78 sigB_1_1 79 spa_1_2 80 sstC_1_1	60	
63	61	gyrB_1_1
64 hemD_1_1 65 hemN_1_1 66 hsdS_1_1 67 hsdS_2_1 68 lip_1_1 69 menC_1_1 70 murC_1_1 71 nuc_1_1 72 pdhD_1_1 73 rpoB_1_1 74 SAV0431_1_1 75 SAV0449_1_1 76 SAV0440_1_1 77 SAV0441_1_1 78 sigB_1_1 79 spa_1_2 80 sstC_1_1	62	hemB_1_1
65 hemN_1_1 66 hsdS_1_1 67 hsdS_2_1 68 lip_1_1 69 menC_1_1 70 murC_1_1 71 nuc_1_1 72 pdhD_1_1 73 rpoB_1_1 74 SAV0431_1 1 75 SAV0440_1_1 76 SAV0441_1_1 77 spa_1_2 80 sstC_1_1	63	
66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		hemD_1_1
66       hsdS_1_1         67       hsdS_2_1         68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1	65	hemN_1_1
68       lip_1_1         69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		hsdS_1_1
69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		
69       menC_1_1         70       murC_1_1         71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1		lip_1_1
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71       nuc_1_1         72       pdhD_1_1         73       rpoB_1_1         74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1	70	
73	71	
73	72	
74       SAV0431_1_1         75       SAV0439_1_1         76       SAV0440_1_1         77       SAV0441_1_1         78       sigB_1_1         79       spa_1_2         80       sstC_1_1	73	rpoB_1_1
76	74	
76	75	
77 SAV0441_1_1  78 sigB_1_1  79 spa_1_2  80 sstC_1_1	76	
79	77	SAV0441_1_1
79	78	sigB_1_1
80 sstC_1_1		spa_1_2
		sstC_1_1
	81	tag_1_1

SEQ ID NO	Probe
82	tyrA_1_1
83	I-aroC_1_1
84	I-aroA_1_1
85	I-cna_1_1
86	I-ebpS_1_1
87	I-eno_1_1
88	I-fbpA_1_1
89	I-fib_1_1
90	I-fnbB_1_1
91	I-srtA_1_1
92	I-stpC_1_1
93	I-fnbA_1_1
94	I-spa_1_1
95	I-aroE_1_1
96	I-aroF_1_1
97	I-aroG_1_1
98	I-asp23_1_1
99	I-atl_1_1
142	b1169_1_1
143	envZ_1_1
144	fliCb_1_1
145	nfrB_1_1
146	nlpA_1_1
147	pilAe_1_1
148	yacH_1_1
149	yagX_1_1
150	ycdS_1_1
151	yciQ_1_1
152	ymcA_1_1
174	ardeSE0106_1_1
175	ardeSE0107_1_1
176	aroiSE0105_1_1
177	atlE_1_1
178	agrB_1_1
179	agrC_1_1
180	alphSE1368_1_1
181	gad_1_1
182	glucSE1191_1_1
183	hsp10_1_1
184	icaA_1_1
185	icaB_1_1
186	mvaSSepid_1_1
187	nitreSE1972_1_1
188	nitreSE1974_1_1
189	nitreSE1975_1_1
190	oiamtSE1209_1_1
191	ORF1Sepid_1_1
192	ORF3bSepid_1_1
193	qacR_1_1
194	sin_1_1
195	ureSE1861_1_1
196	ureSE1863_1_1
197	ureSE1864_1_1

SEQ ID NO	Probe
198	ureSE1865_1_1
199	ureSE1867_1_1
209	folQShaemolyt_1_1
210	mvaCShaemolyticus_1_1
211	mvaDShaemolyt_1_1
212	mvaK1Shaemolyticus_1_1
213	mvaSShaemolyticus_1_1
214	RNApolsigm_1_1
216	agrB2Stalugd_1_1
217	agrC2Stalugd_1_1
218	agrCStalugd_1_1
219	slamStalugd_1_1
222	RNApolsigmSsapro_1_1
223	RNApolsigmSsapro_1_2
224	msrw1Stwar_1_1
225	nukMStwar_1_1
226	proDStwar_1_1
227	proMStwar_1_1
228	sigrpoStwar_1_1
229	tnpStwar_1_1
231	ARG56_1_1
232	ASL43f_1_1
233	BGL2_1_1
234	CACHS3_1_1
235	CCT8_1_1
236	CDC37_1_1
237	CEF3_1_1
238	CHS1_1_1
239	CHS2_1_1
240	CHS4_1_1
241	CHS5_1_1
242	CHT1_1_1
243	CHT2_1_1
244	CHT4_1_1
245	CSA1_1_1
246	5triphosphatase_1_1
247	AAF1_1_1
248	ADH1_1_1
249	ALS1_1_1
250	ALS7_1_1
251	EDT1_1_1
252	ELF_1_1
253	ESS1_1_1
254	FAL1_1_1
255	GAP1_1_1
256	GNA1_1_1
257	GSC1_1_1
258	GSL1_1_1
259	HIS1_1_1
260	HTS1_1_1
261	HWP1_2_1
262	HYR1_1_1
263	INT1a_1_1

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SEQ ID NO	Probe
264	KRE15f_1_1
265	KRE6_1_1
266	KRE9 1 1
267	MIG1_1_1
268	MLS1_1_1
269	MP65_1_1
270	NDE1_1_1
271	PFK2_1_1
272	PHR1_1_1
273	PHR2 1 1
274	PHR3 1 1
275	PRA1_1_1
276	PRS1_1_1
277	RBT1_1_1
278	RBT4_1_1
279	RH01_1_1
280	RNR1_1_1
281	RPB7_1_1
282	RPL13_1_1
283	RVS167_1_1
284	SHA3_1_1
285	SKN1_1_1
286	SRB1_1_1
287	TCA1_1_1
288	TRP1_1_1
289	YAE1_1_1
290	YRB1_1_1
291	YST1exon2_1_1
308	arcA_1_1
309	arcC_1_1
310	bkdA_1_1
311	cad_1_1
312	camE1_1_1
313	csrA_1_1
314	dacA 1 1
315	dfr_1_1
316	dhoD1a_1_1
317	ABC-eltA_1_1
318	agrBfs_1_1
319	agrCfs_1_1
320	dnaE_1_1
321	ebsA_1_1
322	ebsB_1_1
323	eep_1_1
324	efaR_1_1
325	gls24_glsB_1_1
326	gph_1_1
327	gyrAEf_1_1
328	metEf_1_1
329	mntHCb2_1_1
330	mob2_1_1
331	mvaD_1_1
332	mvaE_1_1

SEQ ID NO	Probe
333	parC_1_1
334	pcfG_1_1
335	phoZ_1_1
336	polC_1_1
337	ptb_1_1
338	recS1_1_1
339	rpoN_1_1
340	tms_1_1
341	tyrDC_1_1
342	tyrS_1_1
377	bglB_1_1
378	bgIR_1_1
379	bglS_1_1
380	efmA_1_1
381	efmB_1_1
382	efmC_1_1
383	mreC_1_1
384	mreD_1_1
385	mvaDEfaecium_1_1
386	mvaEEfaecium_1_1
387	mvaK1Efaecium_1_1
388	mvaK2Efaecium_1_1
389	mvaSEfaecium_1_1
390	orf3_4Efaeciumb_1_1
391	orf6_7Efaecium_1_1
392	orf7_8Efaecium_1_1
393	orf9_10Efaecium_1_1
399	atsA_1_1
400	atsB_1_1
401	budC_1_1
402	citA_1_1
403	citW_1_1
404	citX_1_1
405	dalD_1_1
406	dalK_1_1
407	dalT_1_1
408	acoA_1_1
409	acoB_1_1
410	acoC_1_1
411	ahlK_1_1
412	fimK_1_1
413	glfKPN2_1_1
414	ltrA_1_1
415	mdcC_1_1
416	mdcF_1_1
417	mdcH_1_1
418	mrkA_1_1
419	mtrK_1_1
420	nifF_1_1
421	nifK_1_1
422	nifN_1_1
423	tyrP_1_1
424	ureA_1_1

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425	wbbO_1_1
426	wza_1_1
427	wzb_1_1
428	wzmKPN2_1_1
429	wztKPN2_1_1
430	yojH_1_1
431	liac_1_1
449	cymA_1_1
450	cymD_1_1
451	cymE_1_1
452	cymH_1_1
453	cymI_1_1
454	cymJ_1_1
455	ddrA_1_1
456	fdt-1_1_1
457	fdt-2_1_1
458	fdt-3_1_1
459	gatY_1_1
460	hydH_1_1
461	masA_1_1
462	nasA_1_1
463	nasE_1_1
464	nasF_1_1
465	pehX_1_1
466	pelX_1_1
467	tagH_1_1
468	tagK_1_1
469	tagT_1_1
470	glpR_1_1
471	lasRb_1_1
472	OrfX_1_1
473	pa0260_1_1
474	pa0572_1_1
475	pa0625_1_1
476	pa0636_1_1
477	pa1046_1_1
478	pa1069_1_1
479	pa1846_1_1
480	pa3866_1_1
481	pa4082_1_1
482	pilAp_1_1
483	PilAp2_1_1
484	pilC_1_1
485	PstP_1_1
486	purK_1_1
487	uvrDII_1_1
488	vsmI_1_1_
489	vsmR_1_2
490	xcpX_1_1
523	cap1EStrpneu_1_1
524	cap1FStrpneu_1_1
525	cap1GStrpneu_1_1
526	cap3AStrpneu_1_1

SEQ ID NO	Probe
527	cap3BStrpneu_1_1
528	celAStrpneu_1_1
529	celBStrpneu_1_1
530	cglAStrpneu_1_1
531	cglBStrpneu_1_1
532	cglCStrpneu_1_1
533	cglDStrpneu_1_1
534	cinA_1_1
535	cps14EStrpneum_1_1
536	cps14FStrpneum_1_1
537	cps14GStrpneum_1_1
538	cps14HStrpneum_1_1
539	cps19aHStrpneum_1_1
540	cps19aIStrpneum_1_1
541	cps19aKStrpneum_1_1
542	cps19fGStrpneum_1_1
543	cps23fGStrpneum_1_1
544	dexB_1_1
545	dinF_1_1
546	1760Strpneu_1_1
547	acyPStrpneu_1_1
548	endAStrpneu_1_1
549	exoAStrpneu_1_1
550	exp72_1_1
551	fnlAStrpneu_1_1
552	fnlBStrpneu_1_1
553	fnlCStrpneu_1_1
554	gct18Strpneum_1_1
555	hexB1_1_1
556	hftsHstrpneu_1_1
557	immunofrag1Strpneu_1_1
558	immunofrag2Strpneu_2_1
559	immunofrag3Strpneu_2_1
560	kdtBStrpneu_1_1
561	lysAStrpneu_1_1
562	pcpBStrpneu_1_1
563	pflCStrpneu_1_1
564	plpA_1_1
565	prtA1Strpneu_1_1
566	pspC1Strpneu_1_1
567	pspC2_1_1
568	purRStrpneu_1_1
569	pyrDAStrpneum_1_1
570	SP0828Strpneu_1_1
571	SP0830Strpneu_1_1
572	SP0833Strpneu_1_1
573	SP0837_38Strpneu_1_1
574	SP0839Strpneu_1_1
575	ugdStrpneu_1_1
576	uncC_1_1
577	vicXStrepneu_1_1
578	wchA6bStrpneum_1_1
579	wci4Strpneum_1_1

S80	SEQ ID NO	Probe
S81	580	wciK4Strpneum_1_1
S83	581	
S84	582	wciN6bStrpneum_1_1
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S86		
S87		
S88		
S89		wzy18Strpneum 1 1
S90		
S91		wzy6bStrpneum_1_1
606         cpsAlStrgal 1 1           607         cpsBlStrgal 1 1           608         cpsClStrgal 1 1           609         cpsDlStrgal 1 1           610         cpsElStrgal 1 1           611         cpsGlStrgal 1 1           612         cpsIStragal 1 1           613         cpsIStragal 1 1           614         cpsKStragal 1 1           615         cpsKStragal 1 1           616         cpsYStragal 2 1           617         cpsYStragal 2 1           618         cylBStraga 1 1           619         cylEStraga 1 1           620         cylFStraga 1 1           621         cylHStraga 1 1           622         cylIStraga 1 1           623         cylIStraga 1 1           624         cylKStraga 1 1           625         0487Straga 1 1           626         0488Straga 1 1           627         0493Straga 1 1           628         0495Straga 1 1           630         0500Straga 1 1           631         0502Straga 1 1           632         0504Straga 1 1           633         folDStraga 1 1           634         neuAlStrgal 1 1		
607         cpsB1Strgal_1_1           608         cpsC1Strgal_1_1           609         cpsD1Strgal_1_1           610         cpsE1Strgal_1_1           611         cpsG1Strgal_1_1           612         cpsIStragal_1_1           613         cpsIStragal_1_1           614         cpsKStragal_1_1           615         cpsMStragal_1_1           616         cpsYStragal_1_1           617         cpsYStragal_1_1           618         cylBStraga_1_1           619         cylEStraga_1_1           620         cylFStraga_1_1           621         cylHStraga_1_1           622         cylIStraga_1_1           623         cylIStraga_1_1           624         cylKStraga_1_1           625         048Straga_1_1           626         048Straga_1_1           627         0493Straga_1_1           628         0495Straga_1_1           630         050OStraga_1_1           631         0502Straga_1_1           632         0504Straga_1_1           633         folDStraga_1_1           634         neuB1Strgal_1_1           635         neuB1Strgal_1_1		
608         cpsC1Strgal_1_1           609         cpsD1Strgal_1_1           610         cpsE1Strgal_1_1           611         cpsG1Strgal_1_1           612         cpsIStragal_1_1           613         cpsIStragal_1_1           614         cpsKStragal_1_1           615         cpsMStragal_1_1           616         cpsYStragal_1_1           617         cpsYStragal_1_1           618         cylBStraga_1_1           619         cylEStraga_1_1           620         cylFStraga_1_1           621         cylIStraga_1_1           622         cylIStraga_1_1           623         cylIStraga_1_1           624         cylKStraga_1_1           625         0487Straga_1_1           626         0488Straga_1_1           627         0493Straga_1_1           628         0495Straga_1_1           630         0500Straga_1_1           631         0502Straga_1_1           632         0504Straga_1_1           633         folDStraga_1_1           634         neuAlStrgal_1_1           635         neuB1Strgal_1_1           636         neuC1Strgal_1_1		
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610		
611	h	
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622         cyllStraga_1_1           623         cylStraga_1_1           624         cylKStraga_1_1           625         0487Straga_1_1           626         0488Straga_1_1           627         0493Straga_1_1           628         0495Straga_1_1           629         0498Straga_1_1           630         0500Straga_1_1           631         0502Straga_1_1           632         0504Straga_1_1           633         folDStraga_1_1           634         neuA1Strgal_1_1           635         neuB1Strgal_1_1           636         neuC1Strgal_1_1           637         neuD1Strgal_1_1           638         recNStraga_1_1           639         ileSStraga_1_1           645         cyclStrpyog_1_1           646         fah_rph_hlo_Strpyog_1_1           647         int_1_1	621	
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626       0488Straga_1_1         627       0493Straga_1_1         628       0495Straga_1_1         629       0498Straga_1_1         630       0500Straga_1_1         631       0502Straga_1_1         632       0504Straga_1_1         633       folDStraga_1_1         634       neuA1Strgal_1_1         635       neuB1Strgal_1_1         636       neuC1Strgal_1_1         637       neuD1Strgal_1_1         638       recNStraga_1_1         639       ileSStraga_1_1         645       cyclStrpyog_1_1         646       fah_rph_hlo_Strpyog_1_1         647       int_1_1	624	cylKStraga_1_1
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632	630	0500Straga_1_1
633       folDStraga_1_1         634       neuA1Strgal_1_1         635       neuB1Strgal_1_1         636       neuC1Strgal_1_1         637       neuD1Strgal_1_1         638       recNStraga_1_1         639       ileSStraga_1_1         645       cyclStrpyog_1_1         646       fah_rph_hlo_Strpyog_1_1         647       int_1_1	631	0502Straga_1_1
634	632	0504Straga_1_1
635       neuB1Strgal_1_1         636       neuC1Strgal_1_1         637       neuD1Strgal_1_1         638       recNStraga_1_1         639       ileSStraga_1_1         645       cyclStrpyog_1_1         646       fah_rph_hlo_Strpyog_1_1         647       int_1_1	633	folDStraga_1_1
636         neuC1Strgal_1_1           637         neuD1Strgal_1_1           638         recNStraga_1_1           639         ileSStraga_1_1           645         cyclStrpyog_1_1           646         fah_rph_hlo_Strpyog_1_1           647         int_1_1		
637       neuD1Strgal_1_1         638       recNStraga_1_1         639       ileSStraga_1_1         645       cyclStrpyog_1_1         646       fah_rph_hlo_Strpyog_1_1         647       int_1_1	635	neuB1Strgal_1_1
638 recNStraga_1_1 639 ileSStraga_1_1 645 cyclStrpyog_1_1 646 fah_rph_hlo_Strpyog_1_1 647 int_1_1		neuC1Strgal_1_1
639 ileSStraga_1_1 645 cyclStrpyog_1_1 646 fah_rph_hlo_Strpyog_1_1 647 int_1_1		
645		
646 fah_rph_hlo_Strpyog_1_1 647 int_1_1		ileSStraga_1_1
647 int_1_1		
		fah_rph_hlo_Strpyog_1_1
649 in+21F F 1 1		
	648	int315.5_1_1
649 murEStrpyog_1_1		murEStrpyog_1_1
650 oppA_1_1		
651 oppCStrpyog_1_1	651	oppCStrpyog_1_1

SEQ ID NO	Probe
652	oppD_1_1
653	SPy0382Strpyog_1_1
654	SPy0390Strpyog_1_1
655	SpyM3_1351_1_1
656	vicXStrpyog_1_1
687	573Stprmut_1_1
688	580SStprmut_1_1
689	581_582SStprmut_1_1
690	584SStprmut_1_1
691	dltAStrmut_1_1
692	dltBStrmut 1 1
693	dltCppx1Strmut_1_1
694	dltDStrmut_1_1
695	lichStrbov 1 1
696	lytRStprmut_1_1
697	lytSStprmut_1_1
698	pepQStrrmut_1_1
699	pflCStrmut_1_1
700	recNStprmut_1_1
701	ytgBStrmut_1_1
706	atfA_1_1
707	atfB_1_1
708	atfC_1_1
709	ccmPrmi1_1_1
710	cyaPrmi_1_1
711	aad_1_1
712	flfB_1_1
713	flfD_1_1
714	flfN_1_1
715	flhD_1_1
716	floA_1_1
717	ftsK_1_1
718	gstB_1_1
719	hemCPrmi_1_1
720	hemDPrmi_1_1
721	hev_1_1
722	katA_1_1
723	lpp1_1_1
724	menE_1_1
725	mfd_1_1
726	nrpA_1_1
727	nrpB_1_1
728	nrpG_1_1
729	nrpS_1_1
730	nrpT_1_1
731	nrpU_1_1
732	pat_1_1
733	pmfA_1_1
734	pmfC_1_1
735	pmfE_1_1
736	ppaA_1_1
737	rsbA_1_1
738	rsbC_1_1

SEQ ID NO	Probe
739	speB_1_1
740	stmA_1_1
741	stmB_1_1
742	terA_1_1
743	terD_1_1
744	umoA_1_1
745	umoB_1_1
746	umoC_1_1
747	ureR_1_1
748	xerC_1_1
749	ygbA_1_1
776	envZPrvu_1_1
777	frdC_1_1
778	frdD 1 1
779	infBPrvu 1 1
780	lad_1_1
781	tna2_1_1
2843	carO 1 1
2844	gacS_1_1
2845	dhbA_1_1
2846	dhbB_1_1
2847	sid_1_1
2848	csuD_1_1
2849	csuC_1_1
2850	tnp-ACIBA_1_1
2851	waaA-ACIBA_1_1
2852	csuB_1_1
2853	csuA B 1 1
2854	csuA_1_1
2855	put1_1_1
2856	por_1_1
2857	abc_1_1
2858	furACIBA_1_1
2859	dec_1_1
2860	cysI_1_1
2861	trpE_1_1
2862	put3_1_1
2863	ompA-ACIBA_1_1
2902	coa_3_1
2903	coa_2_2
2876	asr_1_1
2877	lacZ_1_1
2878	ehuS_1_1
2879	ehuV_1_1
2880	slyA_1_1
2881	ORF165_1_1
2882	ehuU_1_1
2883	ehuT_1_1
2884	ORF295_1_1
2885	ehuA_1_1
2886	ORF400_1_1
2887	H+ATPase_1_1
2889	smeE_1_1

SEQ ID NO	Probe
2890	eE_1_1
2891	StmPr1_1_1
2892	eD_2_1
2893	ppi_1_1
2894	pmp-STEMA_1_1
2895	pam_1_1
2896	ORF4-STEMA_1_1
2897	ORF2-STEMA_1_1
2898	et_1_1
2899	eF_1_1
2900	StmPr2_1_1
2901	smeF4494_1_1
2904	fasCAXStrdysg_1_1
2906	ydhK_1_1

# b) virulence probes

SEQ ID NO	Probe
100	bsaE_1_1
101	bsaG_1_1
102	cap5h_1_1
103	cap5i_1_1
104	cap5j_1_1
105	cap5k_1_1
106	cap8H_1_1
107	cap8I_1_1
108	cap8J_1_1
109	cap8K_1_1
110	I-hld_1_1
111	I-hysA_1_1
112	I-IgGbg_1_1
113	EDIN_1_1
114	eta_1_1
115	etb_1_1
116	hglA_1_1
117	hglA_2_1
118	hglB_1_1
119	hglC_2_1
120	hla_1_1
121	hlb_1_2
122	lukF_1_1
123	lukS_1_1
124	lukS_2_1
125	NAG_1_1
126	sak_1_1
127	sea_1_1
128	seb_1_1
129	sec1_1_1
130	seg_1_1
131	seh_1_1
132	sel_1_1
133	set15_1_1

SEQ ID NO	Probe
134	set6_1_1
135	set7_1_1
136	set8_1_1
137	sprV8_1_1
138	tst_1_1
139	I-sdrC_1_1
140	I-sdrD_1_1
141	I-sdrE_1_1
153	b1202 1 1
154	eae_1_1
155	eltB_1_1
156	escR_1_1
157	escT_1_1
158	escU_1_1
159	espB_1_1
160	fes_1_1
161	fes_2_1
162	fteA_1_1
163	hlyA_1_1
164	hlyB_1_1
165	iucA_1_1
166	iucB_1_1
167	iucC_1_1
168	papG_1_1
169	rfbE_1_1
170	shuA_1_1
171	SLTII_1_1
172	toxA-LTPA_1_1
173	VT2vaB_1_1
200	gcaD_1_1
201	hld_orf5_1_1
202	icaC_1_1
203	icaD_1_1
204	icaR_1_1
205	psm_beta1and2_1_1
206	purR_1_1
207	spoVG_1_1
208	yabJ_1_1
215	lipShaemolyt_1_1
220	fblStalugd_1_1
230	slushABCStalugd_1_1
292	gehAStwar_1_1
293	CCN1_1_1 CDC28_1_1
294	CLN2_1_1
295	CPH1_1_1
296	CYB1_1_1
297	EFG1_1_1
298	MNT1_1_1
299	RBF1_1_1
300	RBF1_2_1
301	RIM101_1_1
302	RIM8_1_1

SEQ ID NO	Probe
303	SEC14_1_1
304	SEC4_1_1
305	TUP1_1_1
306	YPT1_1_1
307	ZNF1CZF1_2_1
343	asa1_1_1
344	asp1_1_1
345	cgh_1_1
346	cylA_1_1
347	cylB_1_1
348	cylI_1_1
349	cylL_cylS_1_1
350	cylM_1_1
351	ace_1_1
352	ef00108 1 1
353	ef00109 1 1
354	ef0011 1 1
355	ef00113 1 1
356	ef0012 1 1
357	ef0022 1 1
358	ef0031 1 1
359	ef0032 1 1
360	ef0040_1_1
361	ef0058_1_1
362	enlA_1_1
363	esa_1_1
364	esp_1_1
365	gelE_1_1
366	groEL_1_1
367	groES_1_1
368	rt1_1_1
369	sala_1_1
370	salb_1_1
371	sea1_1_1
372	sep1_1_1
373	vicK_1_1
374	yycH_1_1
375	yycI_1_1
376	yycJ_1_1
394	entA_entI_1_1
395	entD_1_1
396	entR_1_1
397	oep_1_1
398	sagA_1_2
432	cim_1_1
433	aldA_1_1
434	aldA_2_1
435	hemly_1_1
436	pSL017_1_1
437	pSL020_1_1
438	rcsA_1_1
439	rmIC_1_1
440	rmlD_1_1

SEQ ID NO Probe  441
442 wbbD 1 1 443 wbbM 1 1 444 wbbM 1 1 445 wbdA 1 1 446 wbdC 1 1 447 wztKpn 1 1 448 yibD 1 1 491 aprA 1 1 492 aprE 1 1 493 ctx 1 2 494 algB 1 1 495 algN 1 1 496 algR 1 1 497 ExoS 1 1 498 fpvA 1 1 499 lasRa 1 1 500 lipA 1 1 501 lipH 1 1 502 Orf159 1 2 503 Orf252 1 1 504 pchG 1 1 505 Ph2A 1 1 506 Ph2B 1 1 507 PLC 1 1 508 plcN 1 1 509 plcR 1 1 510 pvdD 1 1 511 pvdF 1 2 512 pyocinS1 1 1 513 pyocinS2 1 1 514 pyocinS2 1 1 515 pys2 1 1 516 pys2 2 1 517 rbf303 1 1 518 rhlA 1 1 520 rhlR 1 1 520 rhlR 1 1 520 rhR 1 1 521 TAPPH 1 1 522 traPPH 1 1 523 rhR 1 1 534 pyocinS2 1 1 535 pys2 1 1 536 rhlA 1 1 537 rhB 1 1 538 rhlA 1 1 539 ligStrpneu 1 1
443 wbbM_1 1 444 wbbN_1 1 445 wbdA 1 446 wbdC 1 1 447 wztKpn_1 1 448 ylbD_1 1 449 aprA_1 1 491 aprA_1 1 492 aprE_1 1 493 ctx_1 2 494 algB_1 1 495 algN_1 1 496 algR_1 1 497 ExoS_1 1 498 fpvA_1 1 499 lasRa_1 1 500 lipA_1 1 501 lipH_1 1 502 Orf159_1 2 503 Orf252_1 1 504 pchG_1 1 506 PhzB_1 1 507 PLC_1 1 509 plcR_1 1 510 pvdD_1 1 511 pvdF_1 2 512 pyocinS1 in 1 514 pyocinS2_1 1 515 pys2_1 1 516 pys2_2 1 517 rbf303_1 1 518 rhlA_1 1 519 pvdR_1 1 519 pvdR_1 1 510 rhlR_1 1 511 pvdR_1 1 512 pyocinS1 in 1 513 pyocl s1 in 1 514 pyocinS2_1 1 515 pys2_1 1 516 pys2_2 1 517 rbf303_1 1 518 rhlA_1 1 520 rhlR_1 1 520 rhlR_1 1 521 TrAP41_1 2 522 toxA_1 1 522 ligaStrpneu_1 1 533 pycl igaStrpneu_1 1 534 pyocinS1 in 1 555 rhlA_1 1 556 pys2_2 toxA_1 1 5592 ligaStrpneu_1 1 592 ligaStrpneu_1 1 593 lytA_1 1
444 wbbN 1 1 445 wbdA 1 1 446 wbdC 1 1 447 wztKpn 1 1 448 yibD 1 1 491 aprA 1 1 492 aprE 1 1 493 ctx 1 2 494 algB 1 1 495 algN 1 1 496 ExoS 1 1 497 ExoS 1 1 498 fpvA 1 1 500 lipA 1 1 501 lipH 1 1 502 Orf159 1 2 503 Orf252 1 1 504 pchG 1 1 506 PhzB 1 1 507 PLC 1 1 508 plcR 1 1 510 pvdD 1 2 511 pvdF 1 2 512 pyocinS1 1 1 514 pyocinS2 1 1 515 pys2 1 1 516 pys2 2 1 517 rbf303 1 1 518 rhlA 1 1 519 ptd 1 1 520 rhlR 1 1 521 TrAP41 1 2 522 toxA 1 529 ligStrpneu 1 1 530 light 1 1 531 light 1 1 541 light 1 1 552 light 1 1 553 light 1 1 554 light 1 1 555 light 1 1 555 light 1 1 556 light 1 1 557 rbf303 1 1 558 rhlA 1 1 559 rhlB 1 1 559 rhlB 1 1 559 rhlB 1 1 550 rhlR 1 1 550 rhlR 1 1 551 TrAP41 1 2 552 toxA 1 1 559 light 1 1 550 rhlR 1 1
445 wbdA_1_1 446 wbdC_1_1 447 wztKpn_1_1 448 yibD_1_1 491 aprA_1_1 492 aprE_1_1 493 ctx_1_2 494 algB_1_1 495 algN_1_1 496 algR_1_1 497 ExoS_1_1 499 lasRa_1_1 500 lipA_1_1 501 lipH_1_1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 505 PhzA_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1 in 1 511 pvdF_1_2 512 pyocinS2_1_1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf30_3_1_1 518 rhiA_1_1 519 rhiB_1_1 520 rhiR_1_1 521 TnAP41_1_2 522 toxA_1_1 529 igaStrpneu_1_1 529 igaStrpneu_1_1 529 igaStrpneu_1_1 529 igaStrpneu_1_1 520 rhiR_1_1 520 rhiR_1_1 520 igaStrpneu_1_1 520 igaStrpneu_1_1 520 rhiR_1_1 520 igaStrpneu_1_1 520 rhiR_1_1 520 rhiR_1_1 520 igaStrpneu_1_1 520 rhiR_1_1 520 igaStrpneu_1_1 520 rhiR_1_1 520 rhiR_1_1 520 igaStrpneu_1_1
446 wbdC_1 1 447 wztKpn 1_1 448 yibD_1.1 491 aprA_1.1 492 aprE_1.1 493 ctx_1.2 494 algB_1.1 495 algN_1.1 496 algR_1.1 497 ExoS_1.1 498 fpvA_1.1 499 lasRa_1.1 500 lipA_1.1 501 lipH_1.1 502 Orf159_1.2 503 Orf252_1.1 506 PhzB_1.1 506 PhzB_1.1 507 PLC_1.1 508 plcN_1.1 509 plcR_1.1 510 pvdD_1.1 511 pvdF_1.2 512 pyocinS1_1.1 514 pyocinS2_1.1 515 pys2_1.1 516 pys2_2.1 517 rbf303_1.1 518 rhlA_1.1 520 rhlR_1.1 521 TnAP41_1.2 522 toxA_1.1 529 ligaStrpneu_1.1 529 ligaStrpneu_1.1 520 rhlR_1.1 521 TnAP41_1.2 522 toxA_1.1 523 liytA_1.1 524 positypeu_1.1 525 ligaStrpneu_1.1 526 ligaStrpneu_1.1 527 rhaP41_1.2 529 ligaStrpneu_1.1 529 ligaStrpneu_1.1 520 rhlR_1.1 520 rhlR_1.1 520 rhlR_1.1 520 rhlR_1.1 520 rhlR_1.1 520 ligaStrpneu_1.1
447 wztKpn_1_1  448 yibD_1_1  491 aprA_1_1  492 aprE_1_1  493 ctx 1_2  494 algB_1_1  495 algN_1_1  496 algR_1_1  497 ExoS_1_1  498 fpvA_1_1  499 lasRa_1_1  500 lipA_1_1  501 lipH_1_1  502 Orf159_1_2  503 Orf252_1_1  504 pchG_1_1  505 PhzA_1_1  506 PhzB_1_1  507 PLC_1_1  508 plcR_1_1  510 pvdD_1_1  511 pydF_1_2  512 pyocinS1_in_1  513 pyocinS1_in_1  514 pyocinS2_i_1  515 pys2_1_1  516 pys2_2_1  517 rbf303_1_1  518 rhlA_1_1  520 rhlR_1_1  521 TapP41_1_2  522 toxA_1_1  523 ligStrpneu_1_1  524 parameters  525 ligStrpneu_1_1  527 TapP41_1_2  528 ligStrpneu_1_1  529 ligStrpneu_1_1  530 ligA_1_1  540 ligH_1_1  551 light_1_1  552 light_1_1  553 light_1_1  554 light_1_1  555 light_1_1  555 light_1_1  556 light_1_1  557 rbf303_1_1  558 rhlA_1_1  559 light_1_1  559 light_1_1  550 rhlR_1_1
448 yibD_11 491 aprA_1 492 aprE_1 493 ctx_1_2 494 algB_1_1 495 algN_11 496 algR_1_1 497 ExoS_1_1 498 fpvA_1_1 499 lasRa_1_1 500 lipA_1_1 501 lipH_1 1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1im_1 1 514 pyocinS2_1_1 516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 520 rhlR_1_1 521 pyocinS1_1 521 pyocinS1_1 531 prdF_1_1 532 rhlB_1_1 533 prdF33_1_1 534 pyocinS1im_1 1 535 prdF3_1_1 536 prdF3_1_1 537 rbf303_1_1 538 rhlA_1_1 539 rhlB_1_1 530 rhlR_1_1 530 rhlR_1_1 531 pyocinS1_1 531 pyocinS1_1 532 rhlB_1_1 533 pyocinS1_1 534 pyocinS1_1 535 rhlB_1_1 535 rhlB_1_1 536 rhlR_1_1 537 rhlB_1_1 538 rhlA_1_1 539 rhlB_1_1 540 rhlR_1_1 550 rhlR_1_1
491 aprA 1 1 492 aprE 1 1 493 ctx 1 2 494 algB 1 1 495 algN 1 1 496 algR 1 1 497 ExoS 1 1 498 fpvA 1 1 500 lipA 1 1 501 lipH 1 1 502 Orf159 1 2 503 Orf252 1 1 504 pchG 1 1 505 PhzA 1 1 506 PhzB 1 1 507 PLC 1 1 508 plcN 1 1 509 plcR 1 1 510 pvdD 1 1 511 pvdF 1 2 512 pyocin51 1 1 513 pyocin51 1 1 514 pyocin52 1 1 515 pys2 1 1 516 pys2 2 1 517 rbf303 1 1 518 rhlB 1 1 519 rhlB 1 1 520 rhlR 1 1 521 trAP41 1 2 522 toxA 1 1 592 ligaStrpneu 1 1 593 ligaStrpneu 1 1 593 lytA 1 1
492 aprE 1 1 493 ctx 1 2 494 algB 1 1 495 algN 1 1 496 algR 1 1 497 ExoS 1 1 498 fpvA 1 1 499 lasRa 1 1 500 lipA 1 1 501 lipH 1 1 502 Orf159 1 2 503 Orf252 1 1 504 pchG 1 1 505 PhzA 1 1 506 PhzB 1 1 507 PLC 1 1 508 plcN 1 1 509 plcR 1 1 510 pvdD 1 1 511 pvdF 1 2 512 pyocinS1 in 1 1 514 pyocinS2 1 1 515 pys2 2 1 515 pys2 2 1 517 rbf303 1 1 518 rhlA 1 1 519 rhlB 1 1 520 rhlR 1 1 521 TnAP41 1 2 522 toxA 1 1 524 pla 1 524 pla 1 525 pla 1 525 pla 1 526 pla 1 1 527 rbf303 1 1 538 rhlA 1 1 549 rhlB 1 1 559 rhlB 1 1 550 rhlR 1 1
493
494 algB_1_1 495 algN_1_1 496 algR_1_1 497 ExoS_1_1 498 fpvA_1_1 499 lasRa_1_1 500 lipA_1_1 501 lipH_1_1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 505 PhzA_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1im_1_1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf303_1_1 519 rhlB_1_1 520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 593 lytA_1_1 594 nanA_1_1 594
495 algN_1_1 496 algR_1_1 497 ExoS_1_1 498 fpvA_1_1 499 lasRa_1_1 500 lipA_1_1 501 lipH_1_1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_in_1 513 pyocinS1im_1_1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 510 rlR_1_1 520 rhlR_1_1 531 TrAP41_1_2 532 lgaStrpneu_1_1 533 lya anaA_1_1 544 pyocinS1_1_1 555 pys2_1 in_1 551 rbf303_1_1 551 rhf303_1_1 551 rhf303_1_1 551 rhf303_1_1 551 rhlB_1_1 552 rhRAP41_1_2 552 toxA_1_1 559 ligaStrpneu_1_1 559 ranA_1_1 559 ranA_1_1 559 ranA_1_1 559 ranA_1_1
496 algR_1_1 497 ExoS_1_1 498 fpvA_1_1 499 lasRa_1_1 500 lipA_1_1 501 lipH_1 1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 505 PhzA_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1_1_1 514 pyocinS2_1_1 515 pys2_2_1 516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 520 rhRR_1_1 521 TnAP41_1_2 522 toxA_1_1 593 lytA_1_1 594 nanA_1_1
497 ExoS 1_1 498 fpvA_1_1 499 lasRa_1_1 500 lipA_1 1 501 lipH_1_1 502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 505 PhzA_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS2_1_1 514 pyocinS2_1_1 515 pys2_2_1 516 pys2_2_1 517 rbf303_1_1 518 rhIA_1_1 519 rhIB_1_1 520 rhIR_1_1 521 TnAP41_1_2 522 toxA_1_1 593 liytA_1_1 594 nanA_1_1
498
499
S00
501    lipH_1_1   502    Orf159_1_2   503    Orf252_1_1   504    pchG_1_1   505    PhzA_1   506    PhzB_1_1   507    PLC_1_1   508    plcN_1_1   509    plcR_1_1   510    pvdD_1_1   511    pvdF_1_2   512    pyocinS1_1_1   513    pyocinS2_1_1   514    pyocinS2_1_1   515    pys2_1_1   516    pys2_1_1   517    rbf303_1_1   518    rhIA_1_1   519    rhIB_1_1   520    rhIR_1_1   552    toxA_1_1   553    sys_1_1   554    nanA_1_1   554    nanA_1_1
502 Orf159_1_2 503 Orf252_1_1 504 pchG_1_1 505 PhzA1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1im_1_1 514 pyocinS2_1_1 515 pys2_1 1 516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 594 nanA_1_1
503 Orf252_1_1  504 pchG_1_1  505 PhzA_1_1  506 PhzB_1_1  507 PLC_1_1  508 plcN_1_1  509 plcR_1_1  510 pvdD_1_1  511 pvdF_1_2  512 pyocinS1_1_1  513 pyocinS1im_1_1  514 pyocinS2_1_1  515 pys2_1_1  516 pys2_2_1  517 rbf303_1_1  518 rhlA_1_1  519 rhlB_1 1  520 rhlR_1_1  521 TnAP41_1_2  522 toxA_1_1  593 lytA_1_1  594 nanA_1_1
504 pchG_1_1 505 PhzA_1 1 506 PhzB_1 1 507 PLC_1 1 508 plcN_1_1 509 plcR_1 1 510 pvdD_1 1 511 pvdF_1_2 512 pyocinS1_1 1 513 pyocinS1im_1 1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf303_1 1 518 rhlA_1_1 519 rhlB_1 1 520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
505 PhzA_1_1 506 PhzB_1_1 507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1im_1_1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
506 PhzB 1 1 507 PLC 1 1 508 plcN_1 1 509 plcR 1 1 510 pvdD_1 1 511 pvdF 1 2 512 pyocinS1 1 1 513 pyocinS1im 1 1 514 pyocinS2 1 1 515 pys2_1 1 516 pys2_2 1 517 rbf303_1 1 518 rhlA_1 1 519 rhlB_1 1 520 rhlR_1 1 521 TnAP41 1 2 522 toxA_1 1 593 lytA_1 1 594 nanA_1 1
507 PLC_1_1 508 plcN_1_1 509 plcR_1_1 510 pvdD_1_1 511 pvdF_1_2 512 pyocinS1_1_1 513 pyocinS1im_1_1 514 pyocinS2_1_1 515 pys2_1_1 516 pys2_2_1 517 rbf303_1_1 518 rhIA_1_1 519 rhIB_1_1 520 rhIR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
508  plcN 1 1 509  plcR_1 1 510  pvdD_1 1 511  pvdF_1_2 512  pyocinS1_1_1 513  pyocinS2_1_1 514  pyocinS2_1_1 515  pys2_1_1 516  pys2_2_1 517  rbf303_1_1 518  rhlA_1_1 519  rhlB_1_1 520  rhlR_1_1 521  TnAP41_1_2 522  toxA_1_1 592  igaStrpneu_1_1 593  lytA_1_1 594  nanA_1_1
509       plcR_1_1         510       pvdD_1_1         511       pvdF_1_2         512       pyocinS1_1_1         513       pyocinS1im_1_1         514       pyocinS2_1_1         515       pys2_1_1         516       pys2_2_1         517       rbf303_1_1         518       rhIA_1_1         519       rhIB_1_1         520       rhIR_1_1         521       TnAP41_1_2         522       toxA_1_1         592       igaStrpneu_1_1         593       lytA_1_1         594       nanA_1_1
510  pvdD_1_1  511  pvdF_1_2  512  pyocinS1_1_1  513  pyocinS1im_1_1  514  pyocinS2_1_1  515  pys2_1_1  516  pys2_2_1  517  rbf303_1_1  518  rhIA_1_1  519  rhIB_1_1  520  rhIR_1_1  521  TnAP41_1_2  522  toxA_1_1  592  igaStrpneu_1_1  593  lytA_1_1  594  nanA_1_1
511       pvdF 1_2         512       pyocinS1 1_1         513       pyocinS1im_1_1         514       pyocinS2_1_1         515       pys2_1_1         516       pys2_2_1         517       rbf303_1_1         518       rhIA_1_1         519       rhIB_1_1         520       rhIR_1_1         521       TnAP41_1_2         522       toxA_1_1         592       igaStrpneu_1_1         593       lytA_1_1         594       nanA_1_1
512       pyocinS1 1_1         513       pyocinS1im_1_1         514       pyocinS2_1_1         515       pys2_1_1         516       pys2_2_1         517       rbf303_1_1         518       rhIA_1_1         519       rhIB_1_1         520       rhIR_1_1         521       TnAP41_1_2         522       toxA_1_1         592       igaStrpneu_1_1         593       lytA_1_1         594       nanA_1_1
513  pyocinS1im_1_1  514  pyocinS2_1_1  515  pys2_1_1  516  pys2_2_1  517  rbf303_1_1  518  rhIA_1_1  519  rhIB_1_1  520  rhIR_1_1  521  TnAP41_1_2  522  toxA_1_1  592  igaStrpneu_1_1  593  lytA_1_1  594  nanA_1_1
514 pyocinS2_1_1  515 pys2_1_1  516 pys2_2_1  517 rbf303_1_1  518 rhIA_1_1  519 rhIB_1_1  520 rhIR_1_1  521 TnAP41_1_2  522 toxA_1_1  592 igaStrpneu_1_1  593 lytA_1_1  594 nanA_1_1
515       pys2_1_1         516       pys2_2_1         517       rbf303_1_1         518       rhIA_1_1         519       rhIB_1_1         520       rhIR_1_1         521       TnAP41_1_2         522       toxA_1_1         592       igaStrpneu_1_1         593       lytA_1_1         594       nanA_1_1
516 pys2_2_1 517 rbf303_1_1 518 rhlA_1_1 519 rhlB_1_1 520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
517 rbf303_1_1 518 rhIA_1_1 519 rhIB_1_1 520 rhIR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
518       rhIA_1_1         519       rhIB_1_1         520       rhIR_1_1         521       TnAP41_1_2         522       toxA_1_1         592       igaStrpneu_1_1         593       lytA_1_1         594       nanA_1_1
520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
520 rhlR_1_1 521 TnAP41_1_2 522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
522 toxA_1_1 592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
592 igaStrpneu_1_1 593 lytA_1_1 594 nanA_1_1
593   lytA_1_1 594   nanA_1_1
595 nanBStroneu 1 1
JJJ   HalibJupiicu_i_i
596 pcpCStrpneu_1_1
597 ply_1_1
598 prtAStrpneu_1_1
599 pspA_1_2
600 SP0834Strpneu_1_1
601 SP0834Strpneu_1_2
602 sphtraStrpneu_1_1
603 wciJStrpneu_1_1
604 wziyStrpneu_1_1

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SEQ ID NO	Probe
605	wzxStrpneu_1_1
640	CAMPfactor 1 1
641	CAMPfactor_2_1
642	0499Straga_1_1
643	hylStragal_1_1
644	lipStragal_1_1
657	DNaseIStrpyog_1_1
658	fba2Strpyog_1_1
659	fhuAStrpyog_1_1
660	fhuB1Strpyog_1_1
661	fhuDStrpyog_1_1
662	fhuGStrpyog_1_1
663	hylA_1_1
664	hylP_1_1
665	hylp2_1_1
666	oppB_1_1
667	ropB_1_1
668	scpAStrpyog_1_1
669	sloStrpyog_1_1
670	smez-4Strpyog_1_1
671	sof_1_1
672	sof_2_1
673	speA_1_1
674	speB2Strpyog_1_1
675	speCStrpyog_1_1
676	speJStrpyog_1_1
677	srtBStrpyog_1_1
678	srtCStrpyog_1_1
679	srtEStrpyog_1_1
680	srtFStrpyog_1_1
681	srtGStrpyog_1_1
682	srtIStrpyog_1_1
683	srtKStrpyog_1_1
684	srtRStrpyog_1_1
685	srtTStrpyog_1_1
686	vicKStrpyog_1_1
702	hlyXStrmut_1_1
703	igaStrmitis_1_1
704	igaStrsanguis_1_1
705	perMStrmut_1_1
750	flaA_1_1
751	flaD_1_1
752	fliA_1_1
753	hpmA_1_1
754	hpmB_1_1
755	lpsPrmi_1_1
756	mrpA_1_1
757	mrpB_1_1
758	mrpC_1_1
759	mrpD_1_1
760	mrpE_1_1
761	mrpF_1_1
762	mrpG_1_1

SEQ ID NO	Probe
763	mrpH_1_1
764	mrpI_1_1
765	mrpJ_1_1
766	patA_1_1
767	putA_1_1
768	uca_1_1
769	ureDPrmi_1_1
770	ureEPrmi_1_1
771	ureFPrmi_1_1
772	zapA_1_1
773	zapB_1_1
774	zapD_1_1
775	zapE_1_1
782	end_1_1
783	pqrA_1_1
784	urg_1_1
2905	sloStrep_1_1

## c) resistance probes

SEQ ID NO	Probe
785	blaIMP-7_1_1
786	mecISepid_1_1
787	blaOXA-10_1_2
788	blaB_1_1
789	ampC_1_1
790	I-blaR_1_1
791	blaOXA-32_1_1
792	bla-CTX-M-22_1_1
793	pbp2aStrpneu_1_1
794	blaSHV-1_1_1
795	blaOXA-2_1_1
796	blaRShaemolyt_1_1
797	blaIMP-7_1_2
798	I-mecR_1_1
799	blaOXY_1_1
800	dacCStrpyog_1_1
801	femA_1_1
802	mecA_1_1
803	blaIShaemolyt_1_1
804	blavim_1_1
805	pbp2b_1_1
806	pbp2primeSepid_1_1
807	pbp2x_1_1
808	pbp3Saureuc_1_1
809	pbp4_1_1
810	pbp5Efaecium_1_1
811	pbpC_1_1
812	I-mecI_1_1
813	pbp1a_1_1
814	I-blaI_1_1
815	blaTEM-106_1_1

SEQ ID NO	Probe
816	blaOXY-KLOX_1_1
817	ftsWEF_1_1
818	fmhB_1_1
819	cumA_1_1
820	femBShaemolyt_1_1
821	blaPER-1_1_1
822	bla_FOX-3_1_1
823	blaA_1_1
824	psrb_1_1
825	fmhA_1_1
826	mecR1Sepid_1_1
827	blaZ_1_1
828	blaOXA-1_1_1
829	fox-6_1_1
830	blaPrmi_1_1
831	aacA_aphDStwar_1_1
832	aacC1_1_2
833	aacC2_1_1
834	strB_1_1
835	aadA_1_1
836	aadB_1_2
837	aadD_1_1
838	aacA4_1_2
839	strA_1_1
840	aph-A3_1_1
841	aacC1_1_1
842	aacA4_1_1
843	aacA-aphD_1_1
844	I-spc_1_1
845	aphA3_1_1
846	ermC_1_1
847	linB_1_1
848	satSA_1_1
849 850	mdrSA_1_1
851	I-linA_1_1 ermB 1 2
852 853	ermA_1_1 satA_1_1
854	msrA_1_1
855	mphBM_1_1
856	mefA_1_1
857	mrx_1_1
858	dfrStrpneu_1_1
859	dfrA_1_1
860	cmIA5_1_1
861	catEfaecium_1_1
862	cat_1_1
863	tetAJ_1_1
864	tetL_1_1
865	tetM_1_1
866	vanH(tn)_1_1
867	vanA_1_1
868	vanHB2_1_1
	L

SEQ ID NO	Probe
869	vanR_1_1
870	vanRB2_1_1
871	vanS(tn)_1_1
872	vanSB2_1_1
873	vanWB2_1_1
874	ddl_1_1
875	ble_1_1
876	vanXB2_1_1
877	vanY(tn)_1_1
878	vanYB2 1 1
879	vanB_1_1
880	vanZ(tn)_1_1
881	vanC-2_1_1
882	vanX(tn)_1_1
883	acrB_1_1
884	mexB_1_2
885	I-qacA_1_1
886	sulI_1_1
887	sul_1_1
888	cadBStalugd_1_1
889	mexA_1_1
890	acrR 1 1
891	emeA_1_1
892	acrA_1_1
893	rtn_1_1
894	abcXStrpmut_1_1
895	qacEdelta1_1_1
896	elkT-abcA 1 1
897	I-cadA 1 1
898	albA_1_1
899	wzm_1_1
900	msrCb_1_1
901	nov_1_1
902	wzt_1_1
903	wbbl_1_1
904	norA23_1_1
905	mexR_1_1
906	arr2_1_1
907	mreA_1_1
908	I-cadC_1_1
909	uvrA_1_1
910	CRD2_1_1
911	CDR1_1_1
912	CDR1_2_1
913	MET3_1_1
914	FET3_1_1
915	FTR2_1_1
916	MDR1-7_1_1
917	ERG11_1_1
918	SEC20_1_1
2864	aacA4ENCL_1_1
2865	AdeR-ACIBA_1_1
2866	adeA-ACIBA_1_1
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SEQ ID NO	Probe
2867	aac(6p)-lb7_1_1
2868	adeB-ACIBA_1_1
2869	adeC-ACIBA_1_1
2870	AdeS-ACIBA_1_1
2871	blaL2_1_1
2872	blaMIR-3_1_1
2873	ampR_1_1
2874	ampC-ENCL_1_1
2875	blaL1_1_1
2888	sulII_1_1
2907	tetA-ACIBA_1_1
2908	tetR-ACIBA_1_1

## d) controls and utility

SEQ ID NO	Probe
919	rbcL_1_1
925	rbcL_1_2
920	LDHA(hu)_1_1
921	GAPD(hu)_1_1
922	b-Act(hu)_1_1
923	ARHGDIA(hu)_1_1
924	PGK1(hu)_1_1
926	16SPa_1_1
927	23SEfaecium_2_1
928	16SStrepyog_1_1
929	16SStrepneu_1_1
930	16SStrepagalactiae_1_1
931	16SEfaecium_1_1
932	16SEfaecium_2_1
933	16SRNAEf_2_1
934	16SKpn_1_1
935	16SSa_3_1
936	16SRNAEf_1_1
937	16SShominis_1_1
938	16SShaemolyt_1_1
939	23SEfaecium_1_1
940	16SrRNAPrmi_1_1
941	16SrRNAPrvu1_1_1
942	16SSa_1_1
943	16SKlox_1_1
944	p53_1_1
945	0135mihck_1_1
946	FAN_1_1
947	0270cap_1_1
2842	16SStrepdysgal_1_1

The DNA microarray of (1) is preferably suitable for

5 (I) identification of *Staphylococcus aureus* and comprises one or more or all gene probes selected from SEQ ID NO:3-6, 31, 40, 50, 51, 58, 59, 63, 64, 66-69, 71,

74, 76, 77, 79, 2902 and 2903, preferably at least one of the gene probes represented by SEQ ID NO:71, 68, 4 and 69; and/or

(II) identification of *Escherichia coli* and comprises one or more or all gene probes selected from SEQ ID NO:142, 144, 145, 148, 150-152, 160, 161 and 170, preferably at least one of the gene probes represented by SEQ ID NO:145, 160, 161 and 170; and/or

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- (III) identification of *Staphylococcus epidermidis* and comprises gene probes selected from SEQ ID NO:174, 175, 177, 178, 180-182, 185-193, 198 and 199, preferably at least one of the gene probes represented by SEQ ID NO:177, 178 and 190; and/or
- (IV) identification of *Staphylococcus haemolyticus* and comprises one or more or all gene probes selected from SEQ ID NO:211, 213 and 214, preferably at least one of the gene probes represented by SEQ ID NO:211 and 214; and/or
- (V) identification of Staphylococcus lugdunensis and comprises one or more or all gene probes selected from SEQ ID NO:216, 217 and 219-221, preferably at least one of the gene probes represented by SEQ ID NO:216, 219, 220 and 221; and/or (VI) identification of Staphylococcus warneri and comprises one or more or all gene probes selected from SEQ ID NO:224-228 and 230 preferably at least one of the gene probes represented by SEQ ID NO:224, 226 and 230; and/or
- (VII) identification of Staphylococcus saprophyticus and comprises one or more or all gene probes selected from SEQ ID NO:222 and 223; and/or (VIII) identification of Staphylococcus hominis and comprises one or more or all gene probes selected from SEQ ID NO:2096, 194 and 229 (do hybridise with S. hominis DNA) and 211 and 214 (do not hybridise with S. hominis DNA); and/or
- 25 (IX) identification of *Candida albicans* and comprises one or more or all gene probes selected from SEQ ID NO:231-291, preferably at least one of the gene probes represented by SEQ ID NO:232 and 249; and/or
  - (X) identification of *Enterococcus faecalis* and comprises one or more or all gene probes selected from SEQ ID NO:308-310 and 312-342, preferably at least one of the gene probes represented by SEQ ID NO:308, 310 and 314; and/or
  - (XI) identification of *Enterococcus faecium* and comprises one or more or all gene probes selected from SEQ ID NO:377-393, preferably at least one of the gene probes represented by SEQ ID NO:380 and 385; and/or

- (XII) identification of *Klebsiella pneumoniae* and comprises one or more or all gene probes selected from SEQ ID NO:399, 401-404, 408-415, 417, 420-423, 425 and 427-431, preferably at least one of the gene probes represented by SEQ ID NO:401, 410 and 430; and/or
- 5 (XIII) identification of *Klebsiella oxytoca* and comprises one or more or all gene probes selected from SEQ ID NO:459 and 466-469, preferably at least one of the gene probes represented by SEQ ID NO:459, 468 and 469; and/or
  - (XIV) identification of *Pseudomonas aeruginosa* and comprises one or more or all gene probes selected from SEQ ID NO:470-485, 487-493 and 505, preferably at least one of the gene probes represented by SEQ ID NO:471, 474, 488 and 505; and/or

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- (XV) identification of *Streptococcus pneumoniae* and comprises one or more or all gene probes selected from SEQ ID NO:523-591, preferably at least one of the gene probes represented by SEQ ID NO:558 and 562; and/or
- 15 (XVI) identification of *Streptococcus agalactiae* and comprises one or more or all gene probes selected from SEQ ID NO:606-639, preferably at least one of the gene probes represented by SEQ ID NO: 606 and 619; and/or
  - (XVII) identification of *Streptococcus pyogenes* and comprises one or more or all gene probes selected from SEQ ID NO:645-648, 652, 655, 656, 658 and 660, preferably at least one of the gene probes represented by SEQ ID NO:645, 658 and 660; and/or
  - (XVIII) identification of *Streptococcus mutans* and comprises one or more or all gene probes selected from SEQ ID NO:687-701, preferably at least one of the gene probes represented by SEQ ID NO:687, 691 and 692; and/or
- 25 (XIX) identification of *Proteus mirabilis* and comprises one or more or all gene probes selected from SEQ ID NO:706-710, 712-742 and 744-749, preferably at least one of the gene probes represented by SEQ ID NO:721, 725 and 735; and/or (XX) identification of *Proteus vulgaris* and comprises one or more or all gene probes selected from SEQ ID NO:776-778 and 780-781, preferably at least one of the gene probes represented by SEQ ID NO:776, 777 and 781; and/or
  - (XXI) identification of *Acinetobacter baumanii* and comprises one or more or all gene probes selected from SEQ ID NO:2843-2863, preferably at least one of the gene probes represented by SEQ ID NO:2858 and 2863.

In a preferred aspect of present invention, the DNA microarray of embodiment (1) is suitable for species specific identification of at least *S. aureus* and preferably comprises gene probes selected from SEQ ID NO:3-6, 31, 40, 50, 51, 58, 59, 63, 64, 66-69, 71, 74, 76, 77, 79, 2902 and 2903, more preferably from SEQ ID NO:4, 68, 69 and 71, even more preferably comprises at least SEQ ID NO:71.

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In a second preferred aspect, the DNA microarray is suitable for species specific identification of at least *S. aureus, E. coli,* CoNS, Enterococcus sp., and/or Candida sp., and preferably comprises gene probes selected from

- a) SEQ ID NO:4, 68, 69 and 71, preferably SEQ ID NO: 71 for identification of *S. aureus*;
  - b) SEQ ID NO: 145, 160, 161 and 170, preferably SEQ ID NO:145 for identification of *E. coli*;
  - c) SEQ ID NO:177, 178 and 190, preferably SEQ ID NO:178 for identification of *S. epidermidis*;
- d) SEQ ID NO:60, 61, 70, 72, 78 and 125, preferably SEQ ID NO:78 for identification of the genus Staphylococci including *S. aureus*;
  - e) SEQ ID NO:210, 224 and 2906, preferably 2906 for identification of CoNS;
  - f) SEQ ID NO:308, 310 and 314, preferably SEQ ID NO:310 for identification of Enterococcus faecalis;
- 20 g) SEQ ID NO:380 and 385, preferably SEQ ID NO:380 for identification of Enterococcus faecium;
  - h) SEQ ID NO:232 and 249, preferably SEQ ID NO:249 for identification of *Candida albicans*;
- respectively. These microorganisms are the prevalent microorganisms in clinical samples and/or are of the highest diagnostic relevance. The probes listed under (a) to (h) are the most reliable probes for identification of said microorganisms.
  - From above second preferred aspect, there can be selected a set of probes which is even more preferred, namely SEQ ID NO:71, 2906, 145 and 249. A DNA microarray comprising one, several or all of said four probes is suitable for species specific detection or differentiation of

- (i) S. aureus if it comprises SEQ ID NO:71;
- (ii) CoNS if it comprises SEQ ID NO:2906;
- (iii) E. coli if it comprises SEQ ID NO:145; and/or
- (iv) Candida albicans if it comprises SEQ ID NO:249.
- 5 This set of four probes thus forms an especially preferred set of probes for embodiment (1).

There are some further sets of probes which are especially preferred for the DNA microarray of embodiment (1). Namely, there are a few DNA microarrays which form preferred aspects of embodiment (1). They are suitable for species-specific identification and differentiation of the following sets of microorganisms and therefore comprise at least the minimum number of probes which are necessary for the species specific identification:

(A) S. aureus;

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- (B) Staphylococci including S. aureus and CoNS;
- 15 (C) set (A) or (B) additionally including *E. coli*;
  - (D) any of the sets of (A) to (C) additionally including C. albicans;
  - (E) any of the sets of (A) to (D) additionally including Enterococcus sp.;
  - (F) any of the sets of (A) to (E) additionally including Proteus sp. and/or *P. aeruginosa*.
- 20 Sets (B), (C) and (D) are preferred, set (D) is especially preferred.

In addition, the DNA microarray of embodiment (1) may be suitable for additional species specific identification or differentiation of one or more of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Proteus vulgaris*.

In a further especially preferred aspect, the DNA microarray of (1) is suitable for (I) virulence determination of *Staphylococcus aureus* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:100-141; and/or (II) virulence determination of *Escherichia coli* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:153-173; and/or

- (III) virulence determination of *Staphylococcus epidermidis* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:200-208; and/or
- (IV) virulence determination of *Staphylococcus haemolyticus* and comprises the gene probe of group (b) represented by SEQ ID NO:215; and/or
- (V) virulence determination of *Staphylococcus lugdunensis* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:220-221; and/or
- (VI) virulence determination of *Staphylococcus warneri* and comprises the gene probe of group (b) represented by SEQ ID NO:230; and/or
- (VII) virulence determination of *Candida albicans* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:292-307; and/or
- (VIII) virulence determination of *Enterococcus faecalis* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:343-376; and/or
- 15 (IX) virulence determination of *Enterococcus faecium* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:394-398; and/or
  - (X) virulence determination of *Klebsiella pneumonia* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:432-448; and/or
  - (XI) virulence determination of Klebsiella oxytoca; and/or

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- 20 (XII) virulence determination of *Pseudomonas aeruginosa* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:491-522; and/or
  - (XIII) virulence determination of *Streptococcus pneumoniae* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:592-605; and/or
  - (XIV) virulence determination of *Streptococcus agalactiae* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:640-644; and/or
  - (XV) virulence determination of *Streptococcus pyogenes* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:657-686; and/or
    - (XVI) virulence determination of *Streptococcus mutans* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:702-705; and/or

(XVII) virulence determination of *Proteus mirabilis* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:750-775; and/or (XVIII) virulence determination of *Proteus vulgaris* and comprises one or more or all of the gene probes of group (b) selected from SEQ ID NO:782-784.

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In a further especially preferred aspect, the DNA microarray of (1) is suitable for antibiotic resistance determination of (I) Staphylococcus aureus, (II) Escherichia coli, (III) Staphylococcus epidermidis, (IV) Staphylococcus haemolyticus, (V) Staphylococcus lugdunensis, (VI) Staphylococcus warneri, (VIII) Enterococcus faecalis, (IX) Enterococcus faecium, (X) Klebsiella pneumonia, (XI) Klebsiella oxytoca, (XII) Pseudomonas aeruginosa, (XIII) Streptococcus pneumoniae, (XIV) Streptococcus agalactiae, (XV) Streptococcus pyogenes, (XVI) Streptococcus viridans, (XVII) Proteus mirabilis, and/or (XVIII) Proteus vulgaris and comprises one or more or all of the gene probes of group (c) selected from SEQ ID NO:785-909; 2864-2875, 2888, 2907-2908 and/or

it is suitable for antibiotic resistance determination of (VII) *Candida albicans* and comprises one or more or all of the gene probes of group (c) selected from SEQ ID NO:910-918.

In a preferred embodiment, the microarray of (1) is suitable for identification and characterisation, i.e. virulence and/or resistance determination, of the target microorganism and comprises one or more or all of the gene probes of group (a) and additionally one or more or all of the gene probes of group (b) and group (c) for each organism as listed above.

If the identification and/or characterisation of *S. aureus*, *E. coli* and/or *P. aeruginosa* is the aim of a test using the array, then the array comprises preferably at least the core gene probes designated in example 1.7, more preferably all the sequences listed in Tab. 2 and/or Tab. 6. Even more preferred, it consists of said sequences.

The gene probes were considered as most preferable if they were i) known previously to be species-specific, ii) bioinformatically selected to have the least chance to hybridise with nontarget genes and iii) empirically proven to be specific in a series of experiments (see Examples).

In a most especially preferred aspect, the DNA microarray of (1) comprises the following gene probes, even more preferably consists of the following gene probes:

- (I) When the DNA microarray is suitable for identification and characterisation of Staphylococcus aureus, it comprises
- (a) the gene probes represented by SEQ ID NO:3-6, 31, 40, 50, 51, 58, 59, 63, 64, 66-69, 71, 74, 76, 77, 79, 2902 and 2903; and at least one of
- 5 (b) the gene probes represented by SEQ ID NO:100-141 and
  - (c) the gene probes represented by SEQ ID NO:785-909, 2864-2875, 2888, 2907, 2908.
  - (II) When the DNA microarray is suitable for identification and characterisation of *Escherichia coli*, it comprises
- (a) the gene probes represented by SEQ ID NO:142, 144, 145, 148, 150-152, 160,161 and 170; and at least one of
  - (b) the gene probes represented by SEQ ID NO:153-173 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875, 2888, 2907, 2908.
- 15 (III) When the DNA microarray is suitable for identification and characterisation of Staphylococcus epidermidis, it comprises
  - (a) the gene probes represented by SEQ ID NO:174, 175, 177, 178, 180-182, 185-193, 198 and 199; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 200-208 and
- 20 (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875, 2888, 2907, 2908.
  - (IV) When the DNA microarray is suitable for identification and characterisation of Staphylococcus haemolyticus, it comprises
- (a) the gene probes represented by SEQ ID NO:211, 213 and 214; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 215 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- (V) When the DNA microarray is suitable for identification and characterisation of
   Staphylococcus lugdunensis, it comprises
  - (a) the gene probes represented by SEQ ID NO:216, 217 and 219-221; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 220-221 and

- (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- (VI) When the DNA microarray is suitable for identification and characterisation of Staphylococcus warneri, it comprises
- 5 (a) the gene probes represented by SEQ ID NO:224-228 and 230; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 230 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- 10 (VII) When the DNA microarray is suitable for identification and characterisation of Staphylococcus saprophyiticus, it comprises
  - (a) the gene probes represented by SEQ ID NO:222 and 223; and at least one of
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- 15 (VIII) When the DNA microarray is suitable for identification and characterisation of Staphylococcus hominis, it comprises
  - (a) the gene probes represented by SEQ ID NO:2096, 194, 229, 211 and 214; and at least one of
- (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 20 2908.
  - (IX) When the DNA microarray is suitable for identification and characterisation of *Candida albicans*, it comprises
  - (a) the gene probes represented by SEQ ID NO:231-291; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 292-307 and
- 25 (c) the gene probes represented by SEQ ID NO: 910-918, 2864-2875 2888, 2907, 2908.
  - (X) When the DNA microarray is suitable for identification and characterisation of Enterococcus faecalis, it comprises
  - (a) the gene probes represented by SEQ ID NO:308-310 and 312-342; and at least one of
    - (b) the gene probes represented by SEQ ID NO: 343-376 and

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(c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.

- (XI) When the DNA microarray is suitable for identification and characterisation of Enterococcus faecium, it comprises
- (a) the gene probes represented by SEQ ID NO:377-393; and at least one of
- (b) the gene probes represented by SEQ ID NO: 394-398 and
- 5 (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XII) When the DNA microarray is suitable for identification and characterisation of Klebsiella pneumonia, it comprises
  - (a) the gene probes represented by SEQ ID NO:399, 401-404, 408-415, 417, 420-
- 10 423, 425 and 427-431; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 432-448 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- (XIII) When the DNA microarray is suitable for identification and characterisation of 15 *Klebsiella oxytoca,* it comprises
  - (a) the gene probes represented by SEQ ID NO:459 and 466-469; and at least one of
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- 20 (XIV) When the DNA microarray is suitable for identification and characterisation of *Pseudomonas aeruginosa*, it comprises
  - (a) the gene probes represented by SEQ ID NO:470-485, 487-493 and 505; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 491-522 and
- 25 (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XV) When the DNA microarray is suitable for identification and characterisation of Streptococcus pneumoniae, it comprises
  - (a) the gene probes represented by SEQ ID NO:523-591; and at least one of
- 30 (b) the gene probes represented by SEQ ID NO: 592-605 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XVI) When the DNA microarray is suitable for identification and characterisation of Streptococcus agalactiae, it comprises

- (a) the gene probes represented by SEQ ID NO:606-639; and at least one of
- (b) the gene probes represented by SEQ ID NO: 640-644 and
- (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- 5 (XVII) When the DNA microarray is suitable for identification and characterisation of Streptococcus pyogenes, it comprises
  - (a) the gene probes represented by SEQ ID NO:645-648, 652, 655-656, 658 and 660; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 657-686 and
- (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XVIII) When the DNA microarray is suitable for identification and characterisation of *Streptococcus mutans*, it comprises
  - (a) the gene probes represented by SEQ ID NO:687-701; and at least one of
- 15 (b) the gene probes represented by SEQ ID NO: 702-705 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XIX) When the DNA microarray is suitable for identification and characterisation of *Proteus mirabilis*, it comprises
- 20 (a) the gene probes represented by SEQ ID NO:706-710, 712-742 and 744-749; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 750-775 and
  - (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
- 25 (XX) When the DNA microarray is suitable for identification and characterisation of *Proteus vulgaris*, it comprises
  - (a) the gene probes represented by SEQ ID NO:776-778 and 780-781; and at least one of
  - (b) the gene probes represented by SEQ ID NO: 782-784 and
- 30 (c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.
  - (XXI) When the DNA microarray is suitable for identification and characterisation of Acinetobacter baumanii, it comprises
  - (a) the gene probes represented by SEQ ID NO:2843-2863; and at least one of

(c) the gene probes represented by SEQ ID NO: 785-909, 2864-2875 2888, 2907, 2908.

The DNA microarray which is a preferred aspect of embodiment (1) can be fabricated using textbook methods for microarray production, including printing with fine-pointed pins onto the solid support, photolithography using pre-made masks or dynamic micromirror devices, ink-jet printing or electrochemistry on microelectrode arrays (Müller, H.-J., Röder, T., "Der Experimentator: Microarrays, Spektrum Akademischer Verlag, Heidelberg (2004)). Preferred fabrication methods are printing methods spotting the gene probes onto the solid surface of the microarray. The attachment of the spotted DNA to the surface is achieved by covalent or non-covalent binding, preferably by non-covalent binding, more preferably by electrostatic interaction (ionic binding), most preferably by ionic binding of the DNA to amino groups present on the surface of the solid support. Any amino-functionalized microarray support can be used, but gamma aminopropyl silane (GAPS™) coated slides, especially UltraGAPS™ coated glass slides, are preferred in present invention.

The amount of DNA per spot printed onto the array is from 0.1 to 15.0 ng, preferably from 0.1 to 0.2 ng.

Thus, the present invention also pertains to a method for fabrication of a microarray of embodiment (1), which method comprises spotting the gene probes listed above to an appropriate solid support.

The sample of embodiments (1) to (4) may be any sample containing microorganisms, including food samples, environmental samples and clinical specimens. A sample which is a clinical specimen is preferred. The sample or clinical specimen of embodiments (1) to (4) is preferably selected from the group consisting of whole blood, serum, urine, saliva, liquor, sputum, punktate, stool, pus, swabs, wound fluid and positive blood cultures, more preferably is whole blood or a positive blood culture, most preferably is a positive blood culture. If blood culture is used as DNA source, 0.5 ml positive blood culture is sufficient for identification and characterisation of the microorganisms and bacteria present without prior amplification of the target DNA.

Thus, the microarray of present application is

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- (i) a robust diagnostic tool, detecting all tested bacterial reference strains and clinical isolates;
- (ii) sensitive enough to yield positive signals with e.g. only 20 ng of purified genomic S. aureus DNA or 2  $\mu$ g of DNA extracted from blood culture which contains a high percentage of human DNA;
- (iii) highly specific, distinguishing e.g. *S. aureus* from distantly related gramnegative bacteria like *Escherichia coli* or *Pseudomonas aeruginosa* as well as from closely related CoNS;
- (iv) precise enough to identify virulence factors and antibiotic resistance determinant genes without previous amplification by PCR.
  - Moreover, the whole procedure can be accomplished the same day after blood cultures become positive (e.g. in the Bactec<sup>®</sup>). Rapid identification of the causative pathogen in fungemia, bacteremia and sepsis is crucial for several reasons:
- (i) appropriate antimicrobial therapy should be started as early as possible and unnecessary treatment avoided;
- (ii) the prognosis of the patients with sepsis may be improved; and

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(iii) expenditures on antimicrobials and prolonged hospitalisation can be reduced.

The DNA microarray of embodiment (1) is especially suitable for diagnosis of

- (i) bacteremia, fungemia or sepsis, wherein the device preferably comprises probes
   for species specific identification of at least *S. aureus, E. coli,* CoNS, Enterococcus sp., and Candida sp.;
  - (ii) respiratory tract infections, wherein the device preferably comprises probes for species specific identification of at least Candida sp., *S. aureus* and *P. aeruginosa*; and/or
- 25 (iii) urinary tract infections, wherein the device preferably comprises probes for species specific identification of at least *E. coli*, Enterococci sp., Candida sp. and Proteus sp..

With the gene-segment based microarray of (1) there is an excellent correlation between genotypic detection of antibiotic resistance determinants and phenotypic typing using conventional susceptibility testing. In one aspect of the invention, the detection of the resistance genes *mecA*, *blaZ*, *ermA*, *ermC*, *msrSA*, *aadD* and *aacA-aphD* by microarray hybridisation allows for reliable prediction of oxacillin, penicillin, erythromycin, tobramycin and gentamicin resistance in a single assay.

By microarray hybridisation according to present invention it is furthermore possible to discriminate multi-resistant and multi-susceptible MRSA (strain MW2). Multi-susceptible MRSA have been shown to be susceptible to tobramycin and erythromycin (Polyzou, A. et al., J. Antimicrob. Chemother. 48:231-4 (2001); Pournaras, S. et al., J. Clin. Microbiol. 39:779-81 (2001)).

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In a preferred aspect of the invention, simultaneous comprehensive resistance genotyping for oxacillin, macrolide and aminoglycoside resistance genes (preferably *mecA*, *aadD*, *aacA-aphD*, *ermA*,*B*,*C* and *msrSA*) by microarray hybridisation allows the rapid discrimination of multi-resistant or multi-susceptible strains and in consequence other therapeutic options with e.g. macrolides and may reduce reliance on vancomycin (Polyzou, A. et al., J. Antimicrob. Chemother. 48:231-4 (2001); Pournaras, S. et al., J. Clin. Microbiol. 39:779-81 (2001)).

One preferred aspect of embodiment (1) is a DNA microarray for the identification and characterisation of the three important bacteremia causing species Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa in a sample, preferably in blood culture. The microarray allows simultaneous species identification and detection of important virulence and antibiotic resistance genes in a single assay. Preferably, this array consists of 2-20 species specific gene probes, 1-20 virulence gene probes and 1-20 resistance gene probes of at least 100 nt length, more preferably of 200-800 nt length. One especially preferred embodiment is an array comprising or consisting of the gene probes listed in Tab. 2. The probes may be amplified from recombinant plasmids or synthesized by any other method know in the art. These probes represent genes encoding house-keeping proteins, virulence factors and antibiotic resistance determinants. Evaluation with 42 clinical isolates, 3 reference strains and 13 positive blood cultures revealed that this DNA microarray is highly specific in identifying S. aureus, E. coli and P. aeruginosa strains and in discriminating them from closely related Gram-positive and Gramnegative bacterial strains also known to be etiological agents of bacteremia. In Example 1.6 and 1.7, this array was successful in identifying all tested 27 E. coli, P. aeruginosa and S. aureus strains and in discriminating them from 21 closely related Gram positive and Gram negative bacterial strains. There is a nearly perfect correlation between genotypic antibiotic resistance by hybridisation to the S. aureus resistance gene probes mecA (oxacillin/methicillin resistance), aacA-aphD (gentamicin resistance), *ermA* (erythromycin resistance) and *blaZ* (penicillin resistance) and the *E. coli* resistance gene probes *blaTEM-106* (penicillin resistance) and *aacC2* (aminoglycoside resistance) and phenotypic antibiotic resistance determined by conventional susceptibility testing (Example 1.10).

One further preferred aspect of embodiment (1) of the invention is a DNA microarray for the identification and characterisation of *S. aureus* in a sample, preferably in blood culture. Evaluation with 10 clinical isolates, 6 reference strains and 10 positive blood cultures revealed that this DNA microarray is highly specific in identifying *S. aureus* and in discriminating them from closely related Grampositive and Gram-negative bacterial strains also known to be etiological agents of bacteremia (Example 1.11).

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The DNA microarray is - in the context of embodiment (2) - preferably used for *in vitro* differentiation of a plurality of different microbial strains contained in one sample and/or for species-specific identification of one or more microbial strain(s) contained in a mixture of a plurality of microorganisms. The DNA microarray of embodiment (1) is advantageous for this kind of use, as it allows the simultaneous determination of the presence or absence in the analysed sample of all those microbial strains for which the device comprises species specific probes. The array is also suitable for identification and determination of single or of a selection of microbial strains in a mixture of strains, especially in a clinical sample containg additional component, without prior isolation of the target strain. These advantages (simultaneous determination and applicability to clinical samples and mixtures) make the DNA microarray of embodiment (1) superior to conventional techniques of DNA amplification for identification of microbial strains like PCR.

The method of embodiment (3) comprises - after isolating the total DNA (including non-microbial DNA) from a sample - the steps of immediate labelling and microarray-based detection of this isolated DNA with or without, preferably without, further DNA amplification steps after the DNA isolation. It is one advantage of the method (3) that it can be performed without said further DNA amplification steps, i.e. the isolated DNA is labelled and applied to the microarray without prior amplification. The use of a single protocol for all microbial species comprising all steps of a microarray procedure including DNA preparation and DNA-chip hybridisation, is essential for testing blood cultures or other clinical specimens,

where the bacterial diagnosis is usually uncertain. Preferably, a DNA preparation protocol employing sonication for simultaneous cell disruption and target DNA fragmentation is the method of choice to increase the sensitivity of the microarray, in particular towards low-copy number and/or plasmid encoded genes which may be underrepresented in the target DNA.

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The method of embodiment (3) is preferably a method for diagnosis of bacteremia, fungemia or sepsis. Furthermore, the sample or clinical specimen used in embodiment (3) is preferably blood or derived from blood, more preferably is a blood culture. Most preferably, the clinical specimen is a positive blood culture.

To obtain positive signals in the method of embodiment (3), 100 pg of purified genomic microbial DNA may be sufficient (lower detection limit), but preferably at least 1 ng of said DNA should be present in the sample. Usually, at least 10 ng, preferably at least 20 ng, more preferably at least 1 μg of purified genomic microbial DNA or at least 1 μg, preferably at least 2 μg of DNA extracted from blood culture are required. 500 μl of positive blood culture yield enough DNA for several hybridisations.

In a preferred aspect of the method of embodiment (3), the DNA isolated in step (a) is labelled and applied to the analytical device without prior amplification, preferably is labelled by random priming. In a further preferred aspect, the DNA isolated in step (a) is fragmented before the labelling reaction. Both aspects simplify and speed up the analysis in comparison to convention methods.

In the method of embodiment (3), the ratio of microbial DNA to total DNA isolated from said sample or clinical specimen is less than or equal to 100 %, preferably is from 1% to 99%, more preferably from 30 to 60%.

The labelling reaction of the method of embodiment (3) may be any DNA labelling reaction known in the art. However, chemical labelling reactions consisting of chemical attachment of a reporter molecule to the sample DNA and labelling by integration of labelled nucleotides into the sample DNA are preferred. Preferably the reporter molecules are fluorophores, more preferably are of the cyanine group of fluorophores. Most preferably, the DNA is labelled with Cy3, Cy5 and/or Alexa Fluor 647 and Alexa Fluor 546. The ratio of bases to dye molecules (BDR) is preferably less or equal to 60.

The detection of the reporter molecule in the method of embodiment (3) of the invention is preferably done by using a suitable detection system for the bound reporter molecule. This detection system is preferably based on visualization of the reporter molecule, more preferably on fluorescence detection. Furthermore, the detection is preferably done by a microarray scanner or microarray reader.

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In the method of embodiment (3) of the invention, the DNA microarray can be substituted by any other solid support onto which DNA gene probes are attached in a way permitting hybridisation of the DNA in the sample and subsequent detection of the bound DNA. This includes the use of microtiter plates coated with one or several DNA gene probes per well, of glass surfaces (like, e.g., microscopic slides) with DNA spots, of filter paper disks, membranes, gold electrodes and beads (particles with a diameter of from 1 nm to several µm made of glass, plastic, metal etc.) coated with DNA, etc.. The beads are preferably used in a multi-chamber system, more preferably in a microfluidic multi-chamber system, wherein each chamber contains a population of beads. Each bead has an attached DNA sequence and the whole beads population in one chamber will carry the same DNA sequence, each chamber corresponding then to a specific capture probe. The target DNA to be analysed flows through the multi-chamber system and will hybridize with the complementary DNA sequences attached to the beads. Beads could be also attached to a surface by magnetic force, i.e. paramagnetic beads coupled with DNA could be attached on the surface of the magnet and arrange in a lattice structure. Complimentary, beads made of a magnetic material could be attached to an iron surface.

The use of the DNA coated beads or of a DNA microarray of embodiment (1) is preferred. The use of a DNA array is especially preferred.

Thus, in one preferred aspect, in the method of embodiment (3) the analytical device is a DNA microarray. In this case, the detection is preferably performed using a DNA microarray reader. In a second preferred aspect, the analytical device is a DNA coated bead or a set of DNA coated beads (plurality of DNA coated beads). In this case, the application and/or detection step is preferably performed in a microfluidic device.

The kit of embodiment (4) of the invention may additionally comprise reagents for the labelling reactions of embodiment (3) and/or reagents necessary for the hybridisation step of the method of embodiment (3).

The present invention is described in more detail by reference to the following examples. It should be understood that these examples are for illustrative purpose only and are not to be construed as limiting the invention.

#### **Examples**

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In the experimental examples described below, standard techniques of recombinant DNA technology were used that were described in various publications, e.g. Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, or Ausubel et al. (1987), Current Protocols in Molecular Biology 1987-1988, Wiley Interscience. Unless otherwise indicated, all enzymes and kits were used according to the manufacturers' specifications.

#### Example 1.1: Materials and Methods

Reference strains, clinical isolates and culture conditions: Bacterial reference strains were obtained from the American Type Culture Collection (ATCC, Manassas, Va.), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) or the network on antimicrobial resistance in Staphylococcus aureus (NARSA, Herndon, Virginia). Clinical isolates were obtained from the inventors' clinical routine microbiology laboratory.

The following bacteria were used for evaluation of the specificity of the microarray in Examples 1.2-1.10: Staphylococcus aureus (ATCC 25923, NRS123 alias MW2, 5 clinical isolates), Staphylococcus epidermidis (5 clinical isolates), Staphylococcus (clinical isolate), Staphylococcus haemolyticus capitis (clinical isolate). Staphylococcus hominis (clinical isolate), Staphylococcus warneri (clinical isolate), Staphylococcus auricularis (clinical isolate), Micrococcus spp. (clinical isolate), Escherichia coli (ATCC 25922, 6 clinical isolates), Pseudomonas aeruginosa (ATCC27853, 5 clinical isolates), Klebsiella pneumoniae (3 clinical isolates), Proteus mirabilis (2 clinical isolates), Serratia marcescens (2 clinical isolates), Enterobacter cloacae (clinical isolate), Enterobacter aerogenes (clinical isolate), Acinetobacter baumannii (clinical isolate), Stenotrophomonas maltophilia (clinical isolate), Enterococcus spp. (clinical isolate), Enterococcus faecalis (clinical isolate) and

Streptococcus pneumoniae (clinical isolate). Bacterial strains and clinical isolates were grown over night at 37 °C with constant shaking in 5 ml Luria-Bertani (LB) broth or tryptic soy broth (TSB, 30 g/l, Merck) containing 3 g/l yeast extract. Enterococci and streptococci were grown in 10 ml TSB plus yeast without agitation under 5%  $CO_2$ . Overnight cultures were harvested at 2,560 g for 10 min. After discarding the supernatant the pellet was washed in 1 ml TE (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA) and recovered by centrifugation at 17,900 g for 10 min. Cell pellets were used for DNA preparation.

Blood cultures: Aerobic and anaerobic blood culture bottles (BACTEC®, Becton Dickinson, Heidelberg, Germany) were inoculated with blood from patients with suspected sepsis and placed in a BACTEC® 9240 blood culture system (Becton Dickinson), a continuous-reading, automated, and computed blood culture system that detects the growth of microorganisms by monitoring CO<sub>2</sub> production. Incubation was performed according to the manufacturer's recommendations. Bottles with a positive growth index were removed from the incubator, and aliquots of 1 ml of the blood culture suspensions were taken aseptically with a needle syringe. 1 ml-aliquots of the blood culture suspensions were mixed with 1 ml 0.1% Triton®-X-100 and kept at room temperature for 5 min in order to disrupt human blood cells. Bacterial cells were then harvested at 17,900 g for 10 min, pellets were washed in 1 ml TE, recovered by centrifugation and used for DNA preparation. For conventional identification and susceptibility testing, a second 1 ml-aliquot was examined by Gram-stain and subcultured on agar plates. The organisms grown on agar plates were characterised and tested for susceptibility using a VITEK-2 system (bioMérieux, Inc., Nürtingen, Germany), Etest strips (AB BIODISK, Solna, Sweden) or disk diffusion tests following the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (Standards, N.C.f.C.L., Approved standard M2-4a, Villanova, PA (1990)).

For microarray hybridisation experiments, DNA was prepared from 13 blood cultures positive for *S. aureus* (4), *S. epidermidis* (3), *S. pneumoniae* (2), *P. aeruginosa* (1), *E. coli* (2) and *P. mirabilis* (1).

#### Example 1.2: DNA preparation

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Total cellular DNA was extracted and purified either by using the First-DNA Alltissue kit (GEN-IAL GmbH, Troisdorf, Germany) following the instructions of the supplier or by enzymatic lysis followed by phenol/chloroform extraction. For the latter protocol, cell pellets were resuspended in 500 µl lysis buffer (20 mM Tris-HCI, pH 8.0, 2 mM EDTA, pH 8.0, and 1.2% Triton®-X-100) and lysozyme (Sigma, Taufkirchen, Germany) was added to reach a final concentration of 0.8 mg/ml. In addition, lysostaphin (Sigma) was added to a final concentration of 0.2 mg/ml to promote staphylococcal lysis or mutanolysin (0.5 U/µl; Sigma) was added to lyse Streptococci and Enterococci. After incubation at 37°C for one hour, cell lysates were treated with Proteinase K (1 mg/ml; Sigma) for 1 hour at 55°C and then with RNase A (0.2 mg/ml; Qiagen, Hilden, Germany) for 1 hour at 37°C. The volume was increased by the addition of 200 µl TE and the salt concentration was adjusted to 0.7 M by addition of 5 M NaCl. A 10% CTAB (cetyltrimethylammonium bromide) solution in 0.7 M NaCl was added to a final concentration of 1% and incubated at 65°C for 20 min in order to release DNA from polysaccharide DNA complexes. DNA was then extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1) prior to precipitation with one volume of isopropanol. After centrifugation at 17,900 g for 30 min, DNA pellets were washed in 70% ethanol and resuspended in 50-100 µl TE.

Concentration, purity and size of the purified DNA preparations were determined by UV-spectrophotometry (lambda 40, PerkinElmer, Boston USA) and 1% agarose gel electrophoresis.

#### Example 1.3: DNA labelling

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Total DNA from commercially available reference strains, clinical isolates and blood cultures was labelled by a non-enzymatic chemical labelling method using the Label It Cy3/Cy5 kits (Mirus, Madison, USA) or the ULYSIS Alexa Fluor 467 Nucleic Acid Labelling Kit (Molecular Probes; Eugene, USA). Prior to labelling, each target DNA was spiked with three gene segments (1  $\mu$ l each, 30  $\mu$ l) amplified by PCR from selected recombinant plasmids to serve as internal positive controls.

For labelling with the Label It Cy3/Cy5 kit 5  $\mu$ g of high molecular weight DNA (>20 kb) were mixed with 7.5  $\mu$ l reagent in a total volume of 50  $\mu$ l and incubated for 2 hours at 37°C according to the recommendations by the supplier. After adjusting the volume to 200  $\mu$ l with H<sub>2</sub>O and adding 0.1 volume of 5 M NaCl, unbound label was removed by precipitation with 2 volumes of ice-cold absolute ethanol for at least 30 min at -20°C. The labelled DNA was recovered by centrifugation at 17,900

g for 30 min. The pellet was washed with 70% ethanol and resuspended in 70  $\mu$ l TE.

For labelling with the Ulysis Alexa Fluor 647 kit, 1  $\mu$ g DNA was denatured at 95°C for 5 min, cooled on ice, mixed with 20  $\mu$ l labelling buffer and 5  $\mu$ l reagent and incubated at 80°C for 15 min according to the instructions of the manufacturer. Unbound dye was removed by ethanol precipitation as described above. The relative labelling efficiency of a reaction was evaluated by calculating the approximate ratio of bases to dye molecules (acceptable labelling ratios for nucleic acid were  $\leq$ 60). This ratio and the amount of recovered labelled DNA was determined by measuring the absorbance of the nucleic acids at 260 nm and the absorbance of the dye at its absorbance maximum using a lambda40 UV-spectrophotometer (PerkinElmer) and plastic disposable cuvettes for the range from 220 nm to 1,600 nm (UVette; Eppendorf, Hamburg, Germany).

### Example 1.4: Microarray construction

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15 Cloned PCR-products were used to generate probes for the DNA microarray. All together 120 gene segments representing virulence genes, antibiotic resistant determinants and species specific metabolic and structural genes from *S. aureus* (40), *E. coli* (31) and *P. aeruginosa* (49) were represented on the microarray (Tab. 2).

Tab. 2: Gene probes with SEQ ID NOs, function, gi numbers and primer sequences. E. coli gene probes (1-31), P. aeruginosa gene probes (32-80), S. aureus gene probes (81-120).

Ar- ray No.	Sym- bol	Function	gi number	gene probe SEQ ID NO	Primer forward [SEQ ID NO]	Primer reverse [SEQ ID NO]
1	envZ	Inner membrane osmosensor	453286	143		ATCCGCCAGTTGCTT AAC [1234]
2		Enterochelin esterase (siderophore)	145916	161	1	GGCAATAGCTTTCAC CAG [1270]
3		Enterochelin esterase (siderophore)	145916	160	1	CAATAGCTTTCACCA GGG [1268]

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4	nfrB	Bacteriophage N4 receptor, inner membrane protein	16127994	145	ATGGAATTGCGTCT GTTC [1237]	AAGTTTAGCCACAGC AGG [1238]
5	уасН	Putative membrane protein	16127994	148	GACTCGGTACAGC GATTG [1242]	CTGACGTTGGGTATC TCG [1243]
6	yagX	Putative enzyme	16127994	149	CTTTACGACGGTTC TCCC [1244]	AATCTTCCCTGCTGA AATG [1245]
7	ycdS	Putative outer membrane protein	16127994	150	TTGAAACTTCTTAC TGCCG [1246]	AATTTCTAATGCAGC GTATTG [1247]
8	<i>b</i> 1169		16127994	142	GTTTGGGACTTATT GCTCTG [1230]	CATCAGCCACAGTTT CAAG [1231]
9		Putative outer membrane protein	16127994	153	GAATACCAAAGCA GATCGTC [1252]	CCGAGATCGACAACA GAG [1253]
10	fliCb	Flagellar H antigen	8071787	144	ACCACGACAGGTC TTTATG [1234]	AGAGAGGCACCGTC ACTAC [1235]
11	iucA	Aerobactin synthesis (siderophore)	474189	165	CATCAGGCAGTTAT CCTGTC [1276]	AGTCGTCCTCCTGCA TTAC [1277]
12	iucB	Aerobactin synthesis (siderophore)	474189	166	TTCACAGCGGATAT GGAC [1278]	CACTTTGCTCCCAGA AATAC [1279]
13	iucC	Aerobactin synthesis (siderophore)	474189	167	AGACTGGGATTTG GTCAAC [1280]	AGACACCATCCTGCC TTC [1281]
14	papG	Adhesin, P-pili protein	42307	168	GGAGTATATTGCGT GGGTAG [1282]	AAGATTCACCATAGA GGCG [1283]
15	yciQ	Putative membrane protein	16127994	151	ATAGCAGGGCTGT TTGTATC [1248]	GACACGGAAACCAA ATTAAC [1249]
16	ymcA	Hypothetical protein	16127994	152	TATTGTCATCGCGC AGAG [1250]	TGTTGGGTTGAAAGA GTAGC [1251]
17	eae	Genetic locus necessary for the production of attaching and effacing lesions on tissue culture, OM protein adhesin	145852	154	CTAACTCATTGTGG TGGAGC [1254]	CTTGTCATCGGTCAT GTTG [1255]
18	eltB	Enterotoxin subunit B	145830	155	GGCGTTACTATCCT CTCTATG [1256]	TTTCCATACTGATTG CCG [1257]
19	escR	Secretion	2897961	156	TTTGTTGTTATTGG TACTTCATTC [1258]	ATCGAAATTGTTACT GGCG [1259]
20	escT	Secretion	2897961	157	ATAGTAG [1260]	GAATACGTTTAGTTG AGGCG [1261]
21	escU	Secretion	2897961	158	AAGTGAAGAGGTA ATGGCTG [1262]	TACCATCAGTATCCT TGGC [1263]

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22	espB	Protein secreted by enteropathoge nic E. coli	1657262	159	GATGGTGACTCTAT TGCAGG [1264]	CCATACGATTCTGGA CCTC [1265]
23	hlyA	Enterohemorrh agic Escherichia coli hemolysin	525328	163	CTTGGAAATGTTGG TAAAGC [1272]	TAAACTCCTTCGGTT GAGC [1273]
24	hlyB	Enterohemorrh agic Escherichia coli hemolysin	1247757	164	TCAATGCTGAAACT ATAAGGC [1274]	ACTTAGCACCCAGTT CGAC [1275]
25	SLTII	Shiga-like toxin type II	304950	171	TTCTTCGGTATCCT ATTCCC [1288]	TGTGAGGTCCACTTC TTCC [1289]
26	toxA- LTPA	Subunit A of heat-labile enterotoxin	148027	172	AAATGGCGACAAAT TATACC [1290]	CTGGGTCTCCTCATT ACAAG [1291]
27	VT2va B	Verotoxin-2 variant, beta- subunit, shiga- like toxin	148261	173	AAGAAGATGTTTAT GGCGG [1292]	GATTCACAGGTACTG GATTTG [1293]
28	aacC2	aminoglyco- side-(3)-N- acetyltrans- ferase	45769	833	1	CGAAATGCTTCTCAA GATAGG [2613]
29		Class A beta- lactamase	21464484	815		TCTCAGCGATCTGTC TATTTC [2577]
30	strB	Streptomycin resistance protein B	17129524	834	AAGTTTCATTGCCA GACG [2614]	TAGACTGCGTTGCTC CTC [2615]
31		Dihydropteroat e synthase, sulfonamide resistance	17129524	887	1	AATTCTTGCGGTTTC TTTC [2721]
32	algB	Alginate biosynthesis (exopolysacch aride)	150990	494	GCCTC	GAGGATGAGGATGT TGGC [1935]
33	algN	Alginate biosynthesis (exopoly- saccharide)	150999	495	GACTGGCTGAATC GTCTC [1936]	GCAGGTCGTACCAG GAAG [1937]
34	algR	Alginate biosynthesis (exopoly- saccharide)	151003	496		TTCAGGTAGAGCTG GAAATG [1939]
35	IANTA I	Alkaline protease	45279	491		CGACGAAGTGGATA TTGG [1929]
36	aprE	Alkaline	45279	492	GGTCAAGCACATC	ACTTCCTTGCGGTAC TCC [1931]

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37	glpR	Repression of glycerol metabolic enzymes (glp=glycerol-3-phosphate)	1399486	470	CAAGCACAACAAG AAATACG [1886]	TAGACCTCCGAAGA GTTGC [1887]
38	lasRa	Elastase, virulence protein	309873	499	CTGGGACGTTAGT GTCATC [1944]	GTCTTGGCATTGAGT TCG [1945]
39	lasRb	Transcriptional activator of elastase	151325	471	GAGCGACCTTGGA TTCTC [1888]	ATAAGACCCAAATTA ACGGC [1889]
40	lipA	Extracellular triacylglycerol lipase	45340	500	AAGAAGTCTCTGCT CCCC [1946]	ACGATTTCCTCCACC TGT [1947]
41	lipH	Lipophilic protein necessary for the expression of active lipase	483463	501	ATGGCAGTTTCAGT GTCG [1948]	CGAAATAGTCGTCCA GCC [1949]
42	mexA	Multidrug resistance protein MexA precursor	5616092	889	CTCGACCCGATCTA CGTC [2724]	GTCTTCACCTCGACA CCC [2725]
43	Orf25 2	DnaJ-like protein	4545242	503	GACCTGCTGTTCCA GTTG [1952]	AATTCACGGGTTTTC TCG [1953]
44	OrfX	Regulatory protein, glycerol metabolism	1399486	472	ATGGATGCTCGGG TACTG [1890]	CTCAGCTACAGCCAC GAC [1891]
45	pa026 0	Hypothetical protein	15595198	473	GATCGTCTCTGCCC AGTC [1892]	ACATTGATGGTGTCG TCC [1893]
46	pa057 2	Hypothetical protein	15595198	474	AGGAGAGAACATG AGTCGC [1894]	TCCTTGTCCCAGTAG TTACC [1895]
47	pa104 6	Hypothetical protein	15595198	477	AGGCATCCATCGA GCTAC [1900]	AACGTCCGAGCAGG ATAC [1901]
48		Hypothetical protein	15595198	478	GCGAGGAGGTATT CGACA [1902]	CCCTTCTGCGAGTAG TGTT [1903]
49	pa184	Hypothetical protein	15595198	479		CAGGAACAGGTGCT CGTAG [1905]
50	pa408	Hypothetical protein	15595198	481		GAGCCGTAGGTGTT ATCG [1909]
51	pchG	pyochelin	4325021	504		GTCGAACAACGCGA ACAG [1955]
52	PhzA	Phenazine biosynthesis proteins (low molecular weight toxins)	5616088	505	GTTGAAAGGGTTTA CCGAC [1956]	AATTTCTGCATCGGG TTC [1957]

53	PLC	Phospholipase C (heat labile- hemolysin)	151492	507	GACTTCGCTGTTCG ACTTC [1960]	TCGGTTCGAGTTCAT AGC [1961]
54	plcN	Non-hemolytic phospholipase C	151497	508	GTGTTCCAGGTGTT CGAC [1962]	GATAGACGTTGTCCT TGACC [1963]
55	plcR	Phospholipase C regulation	151499	509	ACAACCTGGAACA GCAACT [1964]	CGACTCTTGCGCGTA TTC [1965]
56	PstP	Phosphoenolpy ruvate-protein phosphotransf erase	4545246	485	GAAGTGAACTCCG CCAAG [1916]	TCGAGCATCATCAGG TAGAC [1917]
57	purK	AIR carboxylase II, purine biosynthesis	1621599	486	TCGAGAAGTCGAT GTTCAAG [1918]	CTTGCCGTAGTGATG CAG [1919]
58	rhIA	Rhamnosyl- transferase involved in rhamnolipid biosurfactant synthesis	452502	518		CTCCAGGTCGAGGA AATG [1983]
59	Irnik	Rhamnolipid regulation	1117916	520	TTCGATTACTACGC CTATGG [1986]	GGTCCATTGCAGGAT CTC [1987]
60	toxA	Exotoxin A precursor	15595198	522	GTGCGCTACAGCT ACACG [1990]	CTTGCCTTCCCAGGT ATC [1991]
61	uvrDII	DNA helicase II UvrD	3249556	487		TGAGGATAGTCCCTT CGC [1921]
62		Autoinducer synthesis protein	695153	488		AATATCTTCATCGCC AGTTG [1923]
63	хсрХ	Secretion protein, translocation of exoproteins across outer membrane	45433	490	ACTG [1926]	TGCAAGGTACTCACC AGC [1927]
64		Exoenzyme S, secreted toxin	13892017	497		GATACTCTGCTGACC TCGC [1941]
65		Ferripyoverdin e receptor	1633044	498	AATGCGATAACCAT CAGC [1942]	CCGTCGTACTGGAA GTTG [1943]
66		Hypothetical protein	15595198	475	AGGAGCAACTGAA GCGAC [1896]	TCTGCCTTTACCCAG GAC [1897]
67	F :	Hypothetical protein	15595198	476	AAGGTTGGCAGGA TCAAC [1898]	CTAGTGGCGAAATTG AACAG [1899]
68	pa386 6	Hypothetical protein	15595198	480	TTCCCTAACGAATG CTGTC [1906]	CGTTGCTCCCTCATA CAC [1907]
69	PhzB	Phenazine biosynthesis proteins (low molecular weight toxins)	5616088	506		TTCTCGTAGTAACCC TCGG [1959]

	1	Type IV pilin,	[	482	1	
70	pilAp	involved in twitching motility and attachment	18535593	402	GCTTTACCTTGATC GAACTG [1910]	TCAATAGAGCCAGTC ACACC [1911]
71	PilAp2	type IV pilin, involved in twitching motility and attachment	21629637	483	TGCCGTGAGTGAA ATCAG [1912]	CGTAGTTGGCTTTCC AGTT [1913]
72	piIC	Pilin biogenesis protein	18535591	484	GGTATCAACCCACT AAAGGTC [1914]	GTCCAGAGCTTCTAC CAGAG [1915]
73	pvdD	Pyoverdine synthetase D (siderophore)	1633044	510	GTCAAGGGTGTTG TCTGC [1966]	CTCTGCACAAACTCA GGG [1967]
74	S1	PyocinS1, bacteriocin	286179	512	CTTCAGTTCCGAGA TGCC [1970]	GTAACGAACGCTATC GGG [1971]
75	pyocin S1im	Immunity protein of pyocin S1	286179	513	ATATACGGAAAAAG AGTTTCTTGAG [1972]	AGCACGCCATTCTTT AACTTC [1973]
76	pyocin S2	PyocinS2	286182	514	TATACGGCTTCAGA CTTTCC [1974]	TGGCATAAGTATTGG CAG [1975]
77	pys2( 1)	PyocinS2	15595198	515	TCGCCAATAAGAAG AAATTG [1976]	AGTGGTACTCGAAG GGTTCT [1977]
78	pys2( 2)	PyocinS2	15595198	516	ATCCAGTATATTCC TGCTCG [1978]	TGCAATTTCTTCTTAT TGGC [1979]
79	rbf30 3	B-band LPS (O-antigen) biosynthesis	836903	517	ATCGTTCTGGTCTT CCTTG [1980]	ACCAAAGAGTGTTGA TAGCC [1981]
80	rhIB	Rhamnosyl- transferase involved in rhamnolipid biosurfactant synthesis	452502	519	AACGCTTTCTCGAT CAGG [1984]	GATACTGTGCGGTTG TGA [1985]
81	femA	Factor essential for methicillin resistance	4929298	801		TCACGCTCTTCATTT AGTTCT [2549]
82	fmhA	Factor essential for methicillin resistance	4574232	825	TGACTTCGGATGA GTTCAAT [2596]	GCTGTTAATTGTTGT TGCTTT [2597]
83	fmhB	Factor essential for methicillin resistance, putative	4574234	818	CTCACCCAAATGGA GATTTA [2582]	CTTGCTTTTCAGATG TTTCC [2583]
84	gyrA	DNA gyrase subunit A	296393	60	AGGCTCGTATGATT GAAAAA [1066]	GGTTTTGAGCACGAT ATGTAG [1067]
85	gyrB	DNA gyrase subunit B	296393	61	TTGGCACAACTGAT AAGACA [1068]	AAAAATCGTTCAAAG TGCTC [1069]

86	hemB	Porphobilinoge ne synthase	2589180	62	ATCATCAGCGACAA TGAGAG [1070]	TTTTAACATCTCGA ACTATATCTAA [1071]
87	hemN	Oxygen- independent coproporphyrin ogen oxidase	14349226	65	TCTTCCATTCTCTC AGTCAAA [1076]	AGACCATGTATGTAG GTGGC [1077]
88	hla	α-Hemolysin	46763	120	•	GTAGCGAAGTCTGG TGAAAA [1187]
89	lip	Lipase	393265	68	TGCATCTTCCATTT TAATAGC [1082]	GTCATTGTCCTTTGT TGGTT [1083]
90	menC	o-Succinyl- benzoic acid synthetase	1255258	69	TTGACAGCTTTGCA TTTTTA [1084]	GGCTTTGTTGCTTTT AATGA [1085]
91		N-acetyl- glucosaminida se	2506026	125	AAGTTGCTCAAATA CAAGCTG [1196]	TGATGTTAGCCCAAT CTACA [1197]
92	norA2 3	Quinolone resistance protein	4115706	904	GGTTACTTGTTGCT GCTTTT [2754]	CGTAATCGCAATCGA AATA [2755]
93	пис	Nuclease	46623	71	TGGCTATCAGTAAT GTTTCG [1088]	GAATCAGCGTTGTCT TCG [1089]
94	rpoB	RNA poly- merase B- subunit	677848	73	TGGAAGACATCGT AAACGTA [1092]	TGGATCAAAGAAACG TGAAT [1093]
95	tag	DNA-3- methyladenine glycosidase	6434027	81	TTTTGATTTATCTTC TGACGG [1108]	CATTCATTTTATTCCC ACCT [1109]
96	16SSa	16S rRNA	46498	942	TCTCTGATGTTAGC GGCGG [2830]	TCAGGCTTTCGCCCA TT [2831]
97	clfB	Clumping factor B	3393010	4	TAGCATAGCAACAA ACAGTGA [954]	GTTTTGACCTGAAGC TGTATC [955]
98	EDIN	Epidermal cell differentiation inhibitor	152997	113	AAAGATAGTTCTAA GATAAATGGTC [1172]	GGCCATTATTGGTCT GTTG [1173]
99	elkT- abcA	Lantibiotic epilancin K7 tranlocator	1841513	896	ATTAGAAATTGCGA CTGGTG [2738]	AGCGTGTCATATCCT TCATC [2739]
100	epiP- bsaP	Biosynthesis of lantibiotic epidermin; serine protease	21204850	58	1	GTCAAACGAGTGCTA ATGGT [1063]
101	geh	Lipase precursor; glycerol ester hyderolase	153019	59	TTCAATAGGCGTG GTGTC [1064]	TTATCTGTCGGTTTC TCTGG [1065]
102	mreA	ABC transporter	7548683	907	TACGATGACACCA GTCTTTG [2760]	ATCGACAAAACGTAC AGGAT [2761]
103	murC	UDP-N- acetylmuramo yl-L-alanine synthetase	2642658	70		GGATATTTCTTTCGT GCTGT [1087]

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104	sak	Staphylokinase	47425	126	TGTTATTATTCTCA TTTTCTTCAAT [1198]	ATGCTCTGATAAATC TGGGA [1199]
105	sea	Enterotoxin A	153120	127	TTTTATTCATTGCC CTAACG [1200]	TTTTCAGAGTTAATC GTTTTATTATC [1201]
106	sec1	Enterotoxin C	46566	129	AATTTTTGGCACAT GATTTA [1204]	CTTTTATGTCTAGTT CTTGAGCTG [1205]
107	etb	Exfoliative toxine B precursor	153011	115	TTTTAGCAGCGTCA ATTTTT [1176]	CTGATCCAGAGTTTC CTACCT [1177]
108	seb	Enterotoxin B	152999	128	CGTAGATGTGTTTG GAGCTA [1202]	CTTGAGCAGTCACCT TTTTC [1203]
109	sstC	Iron transport protein	3724154	80	TGATATTGGAAGAT ATTAGCATAGA [1106]	TGACAATCGCTITAT TCATIT [1107]
110	tst	Toxic shock syndrome toxin	18266750	138	TTTTTATCGTAAGC CCTTTG [1222]	CAATAACCACCCGTT TTATC [1223]
111	aacA- aphD	Bifunctional aminoglyco- side modifying enzyme	3676412	843	AGATTTGCCAGAAC ATGAAT [2632]	TGTTGCATTTAGTCT TTCCA [2633]
112	aadD	Aminoglyco- side acetyl transferase	21623792	837	GCTATTGGTGTTTA TGGCTC [2620]	CTGATTGCTTAACTG CTTCA [2621]
113	aph- A3	3'5'-amino- glycoside acetyl- transferase	1272325	840	GAGAATATCACCG GAATTGA [2626]	GCTCGACATACTGTT CTTCC [2627]
114	blaZ	β-lactamase	1575124	827	TGCTTTAGTTTTAA GTGCATGT [2600]	TCCTTCATTACACTC TTGGC [2601]
115	cat	Chlorampheni- col acetyl- transferase	46651	862	AGAAAATTGGGATA GAAAAGAA [2670]	CTGCAAGGCAACTG GTAT [2671]
116	dfrA	S1 dihydro- folate reductase	3676404	859	CAATTACCTTGGCA CTTACC [2664]	CCCTTTTCTACGCAC TAAAT [2665]
117	ermA	rRNA methylase	13785452	852	CCAGAAAAACCCTA AAGACA [2650]	AAAGAACACGATATT CACGG [2651]
118	ermC	Adenine methylase	4138444	846	ACACAGTCAAAACT TTATTACTTCA [2638]	CAACAAGTITATITT CTGTAGTTT [2639]
119	msrS A	Macrolide antibiotic resistance	3892641	854	GACAGATTTTCGAT CCCTTA [2654]	CCTTTTTGTTTTGAT GCACT [2655]
120	mecA	Penicillin bin- ding protein 2'	13785452	802	AGTTGTAGTTGTCG GGTTTG [2550]	TGAAGTCGCTTTTCC TAGAG [2551]

S. aureus, E. coli and P. aeruginosa genes were selected from the literature and databases, and compared by BLAST analysis to all other sequences available in the

NCBI database. Primers were designed to amplify gene segments of 200-810 bp length and devoid of apparent homology with genes of other bacterial species and *Homo sapiens*. Gene segments were amplified by using the puReTaq Ready-To-Go PCR beads (Amersham Biosciences, Freiburg, Germany) and cloned into the pDrive Cloning Vector (Qiagen, Hilden, Germany) according to the recommendations of the suppliers and transformed into competent *Escherichia coli* (XL-1-Blue) cells using the calcium chloride protocol (Sambrook, J., Russel D.W., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY (2001)).

For quality control purposes, all gene probes were partially sequenced and verified (with the BigDye kit 1.1 and an 377 DNA sequencer; Applied Biosystems, Foster City, USA). All sequences obtained were identical or substantially identical (>90% sequence identity) to those obtained from the database.

For DNA-probe production 120 recombinant plasmids containing *S. aureus*, *E. coli* and *P. aeruginosa* gene segments were used for re-amplification. Amplicons were purified and spotted in 4 replicates per slide on UltraGAPS<sup>TM</sup> Coated Slides (gamma amino propyl silane coated slides, Corning, NY, USA). Approximately 1 nl DNA (with a concentration of about 0.1 to about 0.2 ng/nl) per spot was spotted onto the slide with a Biorobotics Microgrid Microarrayer (Genomic Solutions, Ann Arbor, MI, USA).

## Example 1.5: Hybridisation and scanning

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All experiments described represent dual co-hybridisations of two different target DNA samples labelled respectively with Cy3, Cy5 or Alexa647. After removal of unbound label, Cy3 and Cy5/Alexa647 labelled DNAs were pooled and mixed with 10 µg of Salmon Sperm DNA and 50 µg of poly-A-DNA. The mixture was frozen in liquid nitrogen and lyophilised in the dark. Prior to hybridisation the target DNA was reconstituted in 33 µl H<sub>2</sub>O and 55 µl 2x hybridisation solution (Memorec Biotec GmbH, Cologne, Germany) and chemically denatured with 11 µl denaturation buffer D1 (Mirus) and neutralized with 11 µl buffer N1 (Mirus) according the instructions of the supplier. Hybridisation was automatically performed with a TECAN Hybridisation Station (HS400, TECAN, Salzburg, Austria). The arrays were prewashed at 60°C for 1 min with 0.2% SDS and 4x SSC and prehybridised in 120 µl denatured prehybridisation buffer (Memorec) for 30 min at 60°C at mild agitation. After injection of 110 µl labelled DNA, hybridisation was performed at 60°C for 18 hours at mild agitation. The arrays were washed at 50°C in primary

wash buffer (Memorec) - five cycles of 1 min wash time and 30 s soak time - and in secondary wash buffer (Memorec) - five cycles of 20 s wash time and 30 s soak time -, and finally dried at  $30^{\circ}$ C with  $N_2$  (2.7 bar) for 3 min. Hybridised arrays were scanned with a Scan Array 5000 laser scanner (PerkinElmer). Laser light of wavelengths at 532 and 635 nm was used to excite Cy3 dye and Cy5/Alexa647 dye, respectively. Fluorescent images were analysed by the ImaGene software (BioDiscovery, El Segundo, CA, USA).

#### Example 1.6: Specificity

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In order to allow the simultaneous and rapid identification of *S. aureus*, *E. coli* and *P. aeruginosa* grown in blood culture specimens from septicemic patients, a microarray comprising a set of 40 *S. aureus*, 31 *E. coli* and 49 *P. aeruginosa* gene probes of 200 to 810 bp length was developed (Tab. 2).

The specificity of the DNA-chip was validated firstly (compare Example 1.1) with 45 well characterised clinical isolates and reference strains of the three target species as well as other related bacteria and secondly (compare Example 1.2) with 13 blood cultures from sepsis patients.

In all assays, three PCR-amplified DNA-segments, which had been added to each DNA preparation as a positive control, hybridised with the corresponding probes, indicating that labelling and hybridisation had performed efficiently.

- Hybridisation experiments with *S. aureus*, *E. coli* and *P. aeruginosa* target DNAs, respectively, revealed specific hybridisation with the species-specific gene probes (Fig. 1). There was no cross-hybridisation between the three species with the exception of the *S. aureus* 16S rRNA gene probe (16SSa, Fig. 1C), which hybridised also with *E. coli* and *P. aeruginosa* target DNA.
- Identification of *E. coli*, *P. aeruginosa* and *S. aureus* reference strains, clinical isolates and blood cultures (BC) by microarray analysis corresponded by 100% with the conventional identification results (Fig. 1).

#### Example 1.7: Detection and discrimination

#### Example 1.7A: Detection and discrimination of E. coli

30 All DNA samples from 9 *E. coli* strains hybridised always with seven *E. coli* gene probes (*envZ*, *fes* (1) and (2), *nfrB*, *yacH*, *yagX*, *ycdS*) (Fig. 1A, columns 19 to 27);

in the following these genes are designated as core genes. With 14 *E. coli* gene probes variable hybridisation was observed including the antibiotic resistance gene probes *bla-TEM106*, *sul*, *strB* and *aacC2*. Such a variable hybridisation profile is expected for antibiotic resistance genes since acquired resistance to antimicrobials is strain specific. For 11 *E. coli* virulence gene probes (*eae*, *eltB*, *escR*, *escT*, *escU*, *espB*, *hlyA*, *hlyB*, *SLTII*, *toxA-LTPA*, *VT2vaB*) no hybridisation signals were detected with any of the tested *E. coli* isolates and blood cultures. Since these virulence genes are known to be specific for particular *E. coli* pathotypes (Bekal, S. et al., J. Clin. Microbiol., 41:2113-25 (2003)), it was not surprising that they were not present in the tested strains. The *eae*, *esc* and *esp* genes for example are encoded on a chromosomal pathogenicity island, which is typical for enteropathogenic *E. coli* exhibiting the unique virulence mechanism known as attaching and effacing (AE) (Elliott, S.J. et al., Mol. Microbiol., 28:1-4 (1998)). The alpha-hemolysin (*hly*) operon is encoded on a large plasmid of enterohemorrhagic *E. coli* strains (Schmidt, H. et al., Infect. Immun. 63:1055-61 (1995)).

## Example 1.7B: Detection and discrimination of Pseudomonas aeruginosa

DNA samples obtained from *P. aeruginosa* uniformly hybridised with 32 out of 49 *P. aeruginosa* specific gene segments including the *mexA* gene probe (core genes). Variable hybridisation was observed with 17 probes allowing for discrimination of individual *P. aeruginosa* isolates (Fig. 1B, columns 12 to 18).

## Example 1.7C: Detection and discrimination of S. aureus

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Hybridisation experiments performed with 11 *S. aureus* target DNAs revealed signals in all assays with 16 *S. aureus* gene segments (core genes) (Fig. 1C, columns 1 to 11). Variable hybridisation was observed with 14 *S. aureus* gene probes including the 6 antibiotic resistance gene segments *aadD*, *aacA-aphD*, *blaZ*, *dfrA*, *ermA* and *mecA* and the virulence genes *sak*, *sea*, *sec1* and *EDIN*. The gene probes *geh*, *mreA*, *clfB* and *elkT-abcA* hybridised with 8, 10 (*mreA* and *clfB*) and 6 target DNAs respectively. However, PCR amplification of the four genes was positive for all 11 *S. aureus* target DNAs (not shown) suggesting that the four genes were present in all strains investigated and that these gene probes did not allow reliable detection of the four genes in *S. aureus*.

No hybridisation was observed with 10 probes including the toxin genes *seb, tst* and *etb*. In contrast to the community-acquired, multi-susceptible MRSA strain

MW2 that hybridised to *mecA* and *blaZ* only, all six clinical MRSA strains showed the same multiresistant hybridisation pattern and their DNA hybridised to *ermA* (erythromycin resistance), *mecA* (oxacillin resistance) and the *aadD* gene (tobramycin resistance). As for the majority of multiresistant MRSA strains the *ermA* and *aadD* genes were shown to be located upstream and downstream, respectively, of the *mecA* gene in the *mec* chromosomal region (Chambers, H.F., Clin. Microbiol. Rev., 10:781-91 (1997); Polyzou, A. et al., J. Antimicrob. Chemother., 48:231-4 (2001)). Hybridisation to the core gene probes permitted the identification of *S. aureus*, while hybridisation to antibiotic resistance gene probes allowed for discrimination of strains.

# Example 1.7D: Discrimination of *E. coli*, *P. aeruginosa* and *S. aureus* from related bacterial species

Co-hybridisation experiments performed with related bacterial species confirmed the high specificity of the DNA-chip (Fig. 1): For *S. epidermidis* and all other Coagulase-negative staphylococci, cross-hybridisation was observed only with the *S. aureus* 16S rRNA gene probe (16SSa, Fig. 1C) and several common staphylococcal antibiotic resistance determinants (*aadD*, *aacA-aphD*, *aph-A3*, *blaZ*, *cat*, *dfrA*, *ermA*, *ermC*, *mdrSA*, *mecA*) (Fig. 1C, columns 28 to 36). There was no cross-hybridisation with other metabolic or virulence genes of *S. aureus*.

The *Micrococcus* spp. isolate showed no hybridisation with the DNA-chip (column 53). Streptococci (column 56 to 58) and enterococci (columns 54 and 55) showed hybridisation with the staphylococcal 16S RNA gene probe and once with the staphylococcal *aph-A3* aminoglycoside resistance gene probe (*Enterococcus* spp.) (Fig. 1C). Out of 12 strains of seven Gram-negative species (columns 41 to 52), two hybridised with the *S. aureus* 16S rRNA gene probe (*Klebsiella pneumoniae* and *Proteus mirabilis*, Fig. 1C, columns 41 and 47) and one clinical isolate of *Proteus mirabilis* hybridised with the *E. coli* resistance genes *bla-TEM106* (β-lactam resistance), *sul* (sulfonamide resistance) and *strB* (streptomycin resistance) (Fig. 1A, column 42). *Serratia*, *Stenotrophomonas*, *Acinetobacter* and *Enterobacter* species showed no cross-hybridisation with any gene probe.

#### **Example 1.8: Sensitivity**

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While the majority of *P. aeruginosa* probes allowed unambiguous identification, some probes showed variable hybridisation patterns when microarray hybridisation

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was performed with different target DNA samples prepared from the same isolate (Tab. 3).

<u>Tab. 3:</u> Microarray hybridisation signals obtained with different target DNA preparations of *Pseudomonas aeruginosa* isolates.

Isolate									
		C4242		C3	8853	C3	045	C3	755
DNA amount [ng]	130ª	382ª	1350 <sup>b</sup>	510ª	>2400 <sup>b</sup>	550ª	2950 <sup>b</sup>	1180 <sup>b</sup>	>1600 <sup>b</sup>
BDR <sup>c</sup>	22	75	48	29	30	90	41	139	40
No. of hybridised gene probes <sup>d</sup>	38 (88%)	31 (72%)	43 (100%)	36 (88%)	41 (100%)	34 (89%)	38 (100%)	41 (95%)	43 (100%)

<sup>&</sup>lt;sup>a</sup> Labelled with Alexa647

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Successful hybridisation with strong fluorescent signals depends on efficiency of DNA labelling (ratio of bases per one dye molecule) and amount of labelled DNA. For the different target DNA preparations of four clinical isolates, variable hybridisation was observed with 14 gene probes (*uvrDII*, *vsmI*, *pa1069*, *rhlR*, *rhlA*, *rhlB*, *1046*, *pyocinS*, *pyocinS1im*, *plcR*, *plcN*, *PHZb*, *rbf303* and *pIIAp2*). For example, for three different DNA preparations of isolate C4242, hybridisation to *Pseudomonas*-gene probes varied from 31 to 43 probes, respectively, depending on the labelling efficiency and amount of DNA (Tab. 3). The lowest number of signals was detected with 382 ng target DNA, that, however, showed a high base to dye ratio of 75. Overall, the results suggest that varying amounts of DNA and base to dye ratios influenced the hybridisation results of few gene probes. However, irrespective of the varying quality and quantity of the labelled target DNA, 35 of the 49 *P. aeruginosa* gene probes showed robust hybridisation results in all performed experiments.

Example 1.9: Detection and characterisation of pathogens in blood cultures

<sup>&</sup>lt;sup>b</sup> Labelled with Cy3 or Cy5

<sup>&</sup>lt;sup>c</sup> BDR: Base to dye ratio; number of nucleotides per one dye molecule

<sup>&</sup>lt;sup>d</sup> Number of signals obtained with *P. aeruginosa* capture probes (total 49) after hybridisation with different DNA preparations. The percentage of specific hybridisations is compared to the highest number of signals obtained for each isolate (100%).

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Although DNA prepared from blood cultures comprises a mixture of human and bacterial DNA, the resulting hybridisation signals obtained with DNA from 1 ml positive blood culture allowed a clear and unambiguous characterisation of *S. aureus*, *E. coli* and *P. aeruginosa* present in 13 tested blood specimens (Fig. 1). In accordance to the VITEK2 characterisation, positive BACTEC® cultures were identified by microarray hybridisation as multi-resistant MRSA (Fig. 1C, column 8), penicillin-resistant *S. aureus* (column 9 and 11), multi-susceptible *S. aureus* (column 10), *E. coli* (Fig. 1A, columns 26 and 27), *P. aeruginosa* (Fig. 1B, column18), and discriminated from oxacillin resistant *Staphylococcus epidermidis* (columns 33-35), *Proteus mirabilis* (column 43) and *Streptococcus pneumoniae* (columns 57 and 58).

Example 1.10: Correlation between susceptibility testing and microarray hybridisation of selected antibiotic resistance genes

<u>S. aureus</u>: For 11 Staphylococcus aureus strains and blood cultures, susceptibility results determined by the VITEK2 system, Etest strips and disk diffusion tests were compared with the results of the microarray hybridisation assay for the simultaneous detection of antibiotic resistance genes (Tab. 4). The presence or absence of resistance genes as indicated by microarray hybridisation was confirmed by PCR with gene specific primers (results not shown).

20 <u>Tab. 4:</u> Correlation between phenotypic and genotypic antibiotic resistance for 11 S. aureus isolates and blood cultures.

Hybridisation with mecA/blaZ		
No. pos. No. neg.		
10	0	
0	1	
Hybridisation with mecA		
No. pos.	No. neg.	
7	0	
O	4	
	No. pos.  10  0  Hybridisation No. pos.  7	

c) Erythromycin resistance

Hybridisation with ermA, ermC or msrA

	No. pos.	No. neg.	
6 (resistant)	6	0	
5 (susceptible)	0	5	
d) Tobramycin resistance	Hybridisatio	n with <i>aadD</i>	
	No. pos.	No. neg.	
5 (resistant)	5	О	
6 (susceptible)	0	6	
e) Gentamicin resistance	Hybridisation with aacA-aphD		
	No. pos.	No. neg.	
0 (resistant)	0	0	
11 (susceptible)	0	11	
f) Trimethoprim resistance	Hybridisation with dfrA		
	No. pos.	No. neg.	
1 (resistant)	0	1 <sup>b</sup>	
10 (susceptible)	0	10	

<sup>&</sup>lt;sup>a</sup> Number of strains tested for resistance

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For the *S. aureus* strains there was a 100% correlation between phenotypic resistance to penicillin and hybridisation to the *mecA* and/or *blaZ* gene (both genes confer resistance to penicillin, Tab. 4a). Phenotypic resistance to oxacillin correlated 100% with the hybridisation of the *mecA* gene (Table 4b), between resistance to erythromycin and hybridisation to the erythromycin resistance genes ermA, ermC or msrSA (Tab. 4c) and between resistance to tobramycin and hybridisation to the aadD gene (Tab. 4d). Furthermore, they all showed 100% correlation between phenotypic susceptibility to gentamicin and no hybridisation to the resistance genes aacA-aphD (Tab. 4e). Notably the dfrA gene of the trimethoprim resistant strain MW2 (MIC of 1  $\mu$ g/ml) was not detected by microarray hybridisation (Tab. 4f), whereas PCR amplification revealed the presence of the dfrA gene.

E. coli and other Gram negative bacteria: The prototype microarray harboured only

b dfrA gene detected by PCR

four *E. coli* and one *P. aeruginosa* resistance gene probes which do not yet allow a comprehensive prediction of antibiotic resistances. Nevertheless, hybridisation with the *E. coli* resistance gene probe *blaTEM106* was observed in one *P. mirabilis* and four *E. coli* strains and correlated with phenotypic ampicillin resistance for all five strains (Tab. 5).

<u>Tab. 5:</u> Correlation between ampicillin/penicillin resistance, gentamicin/tobramycin resistance and streptomycin resistance and hybridisation with the resistance gene probes *blaTEM-106*, *aacC2*, *aph-A3* and *strB*, respectively.

Species	Resistance	<u> </u>	Hybridisation with		
	phenotype <sup>a</sup> -	blaTEM-106 <sup>b</sup>	aacC2 <sup>b</sup>	aph-A3 <sup>c</sup>	<i>strB</i> <sup>b</sup>
E. coli ATCC	cuccontible		_		
25922	susceptible	-	-	-	-
E. coli C4821	AMP, STR	+	-	-	+
E. coli F3437	AMP	+	-	-	-
E. coli C3941	AMP, STR	+	-	-	+
E. coli F1806 <sup>d</sup>	AMP, GEN,				
E. COII F1806°	TOB, STR	+	+	+	+
E. coli C4547	AMPi	••	-	-	-
E. coli C4230	AMP	-	-	-	-
E. coli C3940	susceptible	-	-	-	-
E. coli F1642 <sup>d</sup>	STR	-	-	-	+
P. mirabilis C4024	AMP, STR	+	-	-	+
P. mirabilis C4403	susceptible	-	-	-	-
<i>P. mirabilis</i> F1738	susceptible	-	-	-	-

<sup>&</sup>lt;sup>a</sup> AMP, ampicillin; GEN, gentamicin; STR, streptomycin; TOB, tobramycin; i, intermediate

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One *E. coli* blood culture showed also resistance to tobramycin and gentamicin. This phenotypic resistance correlated with the hybridisation of the *aacC2* gene probe for aminoglycoside resistance and the *S. aureus aph-A3* probe for tobramycin/kanamycin resistance (Tab. 5). For one *P. mirabilis* and four *E. coli* 

<sup>&</sup>lt;sup>b</sup> *E. coli* gene probes

<sup>&</sup>lt;sup>c</sup> S. aureus gene probes

<sup>&</sup>lt;sup>d</sup> Positive blood culture

strains, phenotypic resistance to streptomycin correlated with hybridisation to the *strB* probe (Tab. 5).

All *P. aeruginosa* strains hybridised with the *mexA* gene probe (Fig. 1) and showed phenotypic resistance to tetracycline, trimethoprim/sulfamehoxazole, penicillins (ampicillin, mezlocillin) and cephalosporines (cefazolin, cefixime, cefuroxime). The *mexA-mexB-oprM* operon is a determinant for a three component efflux system responsible for intrinsic and acquired multiresistance in *P. aeruginosa* (ß-lactams, fluoroquinolones, trimethoprim, sulphonamides, chloramphenicol and others) (Poole, K., Clin. Microbiol. Infect. 10:12-26 (2004)).

# 10 Example 1.11: Microarray for specific detection of *S. aureus*

#### A) Strains and Cultures

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Reference strains and clinical isolates: The following bacteria were purchased from the American Type Culture Collection (ATCC, Manassas, Va.) or the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DMSZ, Braunschweig, Germany) and were used for evaluation of the specificity of the microarray: *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228; ATCC 18610) *Staphylococcus saprophyticus* (ATCC 14953), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853). Ten clinical MRSA (methicillin resistant *S. aureus*) isolates were obtained from the inventors' clinical routine microbiology laboratory.

<u>Bacterial cultures:</u> Bacterial strains and clinical isolates were plated either onto sheep blood or onto Mueller-Hinton agar from 50% glycerol stocks. One colony was then picked and transferred to 5 ml Luria-Bertani (LB) broth and cultured overnight at 37°C.

Blood cultures: Aerobic blood culture bottles (BACTEC® Plus aerobic, Becton Dickinson, Heidelberg, Germany) were inoculated with 100 CFU of *S. aureus* after adding 10 ml blood from healthy volunteers. A BACTEC® 9240 blood culture system (Becton Dickinson) - a continuous reading, automated, and computed system detecting the growth of microorganisms by monitoring CO<sub>2</sub> production – was used for incubation according to the manufacturer's recommendations. Bottles with a positive growth index were removed from the incubator, and an aliquot of 1 ml of the blood culture suspension was taken aseptically with a needle syringe. The

aliquot was equally divided, with one part for subculture on agar plates and CFU determination, and one part for DNA isolation.

Additionally, in order to test the microarray upon real conditions, samples were collected from ten clinical positive blood culture specimens cultivated under the same conditions as described above. Six of them were positive for different *S. aureus* strains and four for other bacterial species (*Staphylococcus epidermidis*, *Streptococcus mitis*, *E. coli* and *Klebsiella oxytoca*). Blood culture aliquots of 500 µl were used for DNA preparation.

## B) Generation of the S. aureus specific microarray

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About 140 gene segments of *S. aureus* genes, but also a few of CoNS (SEQ ID NO: 177,178,179), were selected from the literature and nucleotide databases in order to cover different functional categories (virulence factors, species-specific metabolic and structural features, antibiotic resistance determinants). Tab. 6 provides the complete list of selected genes with gene symbol, gene function and SEQ ID NO of the segments.

<u>Tab. 6:</u> Selected *S. aureus* genes, selected segments (SEQ ID NO) and primers used for segment amplification (SEQ ID NO)

Gene symbol	Functions	gene probe SEQ ID NO	Primer forward [SEQ ID NO]	Primer reverse [SEQ ID NO]
atl	autolysin	99	AGCTGAGACGACACA AGATCAAA [1144]	TTATATTGCGTTTCAAGA GCTGC [1145]
aroA	3-phosphoshikimate 1-carboxyvinyl- transferase	84	ACCTTCAATATTCGCA TCC [1114]	TATTCCGATTATTAGGCG TAG [1115]
aroC	Chorismatsynthase	83	ATGAGATACCTAACAT CAGGAGAATCA [1112]	GCTATTCTTCCATCTAATT TACGATCATA [1113]
aroE	Shikimatdehydrogen ase	95	GTTATCAATTAATACA ACCCCTGAAGC [1136]	TGGAACTAATTCTCCTTC GATTGTTA [1137]
aroF	3-deoxy-D-arabino- heptulosonate-7- phosphate synthase	96	16 16 11 16 1 1 16 16 1 16 1	ATTACACCATTAACGATA ATTGGCAT [1139]
aroG	Chorismat-Mutase	97	AGACTTATTATCTAAA CGTGGTGAACTAGC [1140]	CAAATGATTTATTGCCGT CTCCTA [1141]
asp23	alkaline shock protein	98	AAAATTGCTGGTATC GCTGCA [1142]	GTCATTACATCATCAACTT GCATGTTA [1143]
cata	catalase	1	TAAATTGTTTAGATTA CAATCAGAGG [948]	TTCAAAGTTTTCGTATGTT TCA [949]

clpC	endopeptidase	7	AATGCTGCTAACCTG CGTGAT [960]	CACGTCTAACCGCTTTAC TGATTG [961]
clpP	endopeptidase	8	AAAGTAAAGAGTAGA CTAAGCTGTCTGCTC [962]	ACCTAATAAAATTCAAGC ATTGGGA [963]
ctaA	cytochrome biosynthesis	9	AAGAATTTAAAATGGT TAGGTGTCGTA [964]	ACGTAATCGTTTTGTTGC CAAATA [965]
ctsR	transcription repressor of class III stress genes homologue	10		TTGCGTTTCTATTTAGCTC AGACA [967]
dltA	D-alanine-D-alanyl carrier protein ligase	11	ACAGAGCAGCAAAAG CGTTAGTG [968]	GACCTTGAATGAACCATT GACCAT [969]
dltB	hypothethecal membrane transporter	12	CATATGGTGATTTAC ATTCTTCTTAATTG [970]	CCTAACCATGTACTTTGT AACACTTTCA [971]
dItC	D-alanyl carrier protein	13	AAATTTATTAGCAGAA GTAGCAGAAAATG [972]	CTGAACTCTTCTAATGCTT CAACGATT [973]
dnaK	Heat-shock-protein	14	TTTAGGCGAAAATATT GGTGAAGA [974]	TITGTCGTCGTCTTTTACT TCGTT [975]
elkT	lantibiotic epilancin K7 translocator	15	1	GAGCGTATCGCATAAATA ATCTTTTC [977]
eno	2-phosphoglycerate dehydrogenase	87	CGATGTTCATCATTGG TACTGGTA [1120]	GGTGTTACTAAAGCAGTT GAAAACG [1121]
glnA	glutamine synthetase; belongs to the femC locus	17	TAGTCACCATGAAGTT GCCCC [980]	CCTCTTGAAGATGGTACA CGGAT [981]
glnR	glutamine synthetase repressor; belongs to the femC locus	18		CACCACGATTTATTGGCA AAGTT [983]
grIA	DNA tensisements	19	TTGAATCACCAAATTG AGGTTGT[984]	CAGTCGTTCAGATTTGAA TTTCTTT [985]
grlB	gyrase-like protein beta subunit B	20	[986]	AAACTTAAAATACTTTCTG AATATTGATCAT [987]
groEL	stress response; heat shock protein	21		TGTTAATGCATCGCCTTC AAC[989]
groES	stress response; heat shock protein	22	ATGTATGTTAGCACTC TITAATGTTAAGTG [990]	GTTTAGTTGTGTTTCATTT TCGTT [991]
gyrA	DNA gyrase subunit A	60	CATCATTAATTCGATT CCCTGAAT [1066]	TCATTTACTTCATCTGCAT CCTCTT [1067]
gyrB	DNA gyrase subunit B	61		AAGATTTGTGGCATATCC TGAGTTA [1069]
hemA	Glutamyl-transfer RNA reductase	23	TGTCATATTATCAACA TGTAATCGAACTG [992]	AATATCAGTAATTCCAGA ACCAAGAAGAT [993]
hemB	Porphobilinogene synthase	62	TTGATAGACATAGAA GATTGAGATCATCAG [1070]	ACTTGAGAAATTGCTGTT TTAACAAGTAG [1071]
hemC	Porphobilinogene deaminase	63	GTAAATTAGTCGTTG	GGGATAGTGGTGTATGTG TTTTAGAAATA [1073]

hemD	Uroporphyrinogene III synthase	64	TGTTGATAACATTGCT GTGATAGGAA [1074]	AATGCATCGATTTGTTGA TGTTCTA [1075]
hemE	Uroporphyrinogene decarboxylase	24	AAAATGATCAAAGGT GAAGAAACATC [994]	AATCCTCGACATTTAATG CACCTAC [995]
hemH	Ferrochelatase	25	AATGGGATTATTAGTT ATGGCTTATGG [996]	GTGGATATGGATCATTAT TCTTTTCG [997]
hemL	GSA-1- Aminotransferase	26	ATGAGATATACGAAAT CAGAAGAAGCA [998]	CTAATCTTAAAGTATCCAA TGTAGCTTCTGTA [999]
hemN	oxygen-independent coproporphyrinogen oxidase		ACAGAATCAACCTGT AGATGAGTACTTAGA T [1076]	TGATATTCGTATAACGCA CACCATC [1077]
hemY	putative involved in a late step of protoheme IX synthesis	27	AAACAGCAAGATCCT AATATTGATGTAAC [1000]	CTCTACGTACAATCGATA CTAATTCATTATCT [1001]
lepA	GTP-binding protein	28		CTATAACCAAAACCTAAT GCTTGTGAC [1003]
IrgA	holin-like protein LrgA	29	AAAGACGCATCAAAA CCAGCA [1004]	GGCTAATGACACCTAAAG AGTTAACAACT [1005]
IrgB	holin-like protein LrgA	30	GATTAACCACTTAGCA CTAAACACACCT [1006]	AATGTTTAACAAGCACTT CACGCT [1007]
lytM	peptidoglycan hydrolase	31	CGACAAACACCCAAC AAGCA [1008]	TGGCTGTTATACGCTTGG TTGT [1009]
menB	naphthoate synthase	32	GTTATCGTATTAACTG GTGAAGGTGATT [1010]	ACATTTAGTACATTACCG CCACCTAC [1011]
menC	o-succinylbenzoic acid synthetase	69	TTTAAGTCACAAATTG TAACACCGAA [1084]	TTAATTTAATTCTGGTCG GCTTTGT [1085]
menD	2-Succinyl-6- hydroxy-2,4- cyclohexadiene-1- carboxylase	33	CGTAAGGGAAGTAGT TATCAGTCCG [1012]	TTAGCTGTATACTCGAAA TCCAATCC [1013]
menE	O-succinylbenzoic acid-CoA ligase	34	ATGGACTTTTGGTTAT ATAAACAAGCAC [1014]	TATTTCAGCAATGTCACC CGTATTA [1015]
menF	Isochorismate- Synthase	35		TCACTATCTGGATCAGAA TCTTTAACAAT [1017]
murC	UDP-N- acetylmuramoyl-L- alanine synthetase	70	CTTGGGGTGATGATG AACATCTA [1086]	AAGTGTGTGGTTGAAATA CTGCAA [1087]
mutL	DNA mismatch repair protein	38	TCGTTTACATCATAAT AATCATCAGAC [1022]	ACACAGAGAATAACCAGG AGAAGA [1023]
mutS	DNA mismatch repair protein	39		TCAAGTTGCGAAATTAGC TGA [1025]
pbg	porphobilinogen synthase	41	GGTGTTCCAAACTCA AAAGATGATATA [1028]	TTGACACCATAACTCATTA TAGGAATATTG [1029]
pdhB	pyruvate dehydro- genase (lipoamide): subunit E1beta	43		TTGGTAACCAAACATTTTC AGCTT [1033]

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pdhC	dihydrolipoamide acetyltransferase: subunit E2	44	CTGGAGATACTATTG AAGAAGACGATG [1034]	TTGCTTTTACAGTTCTGTT TTCATCTAC [1035]
pdhD	dihydrolipoamide dehydrogenase: subunit E3	72	CAGGTAAATTAGTTGT	AGTGGTAAACCTGGAACG ATATCA [1091]
rpoB	RNA polymerase B- subunit	73	ATTGTTACGTGCATTA GGTTTCTCA [1092]	TTTCTACTGGCTCGTCTAT AACGC [1093]
rsbU	putative operon encoding alternate sigma factor	45	TAGTTATCGAGATTAT CAAAGATTGGTAGA [1036]	GTAATTGTGAGTGTCCAT AAGAATCCA [1037]
rsbV	putative operon encoding alternate sigma factor	46	TGAATCTTAATATAGA AACAACCACTCAAG [1038]	ACGATCTGACACACCTAA AATGTA [1039]
rsbW	putative operon encoding alternate sigma factor	47	TCTAAAGAAGATTTTA TCGAAATG [1040]	CCCACATTGTTATTTTCTT TGTAT [1041]
sdrC	serine-aspartate repeat protein multigene family	139		CCTTTATCAATCGCAATG TC [1225]
sdrD	serine-aspartate repeat protein multigene family	140	1	AACTGAAGATAAGCCGTT TG [1227]
sdrE	serine-aspartate repeat protein multigene family	141		GCAAAACAAGATGATGCA ACG [1229]
sgp	G protein	48	TGAGATAGATGCAAT CATGTTTATGG [1042]	GAAATAGGTACAATCTCT GTAAAGTCCATATA [1043]
sigB	sigma factor B	78	i e	CTCTGAAGTCGTGATACA TGCA [1103]
sirR	sit operon metal dependent repressor	49	AATATAATTGGGAAG AAGTACATCAAGAAG [1044]	ATATTAGCAAATCGGTCT TATCTCTCA [1045]
sodA	superoxide dismutase	50	TTGAATTACCAAAATT ACCATACG [1046]	CTCCCAGAATAATGAATG GTTTAAAT [1047]
sodB	superoxide dismutase	51	GCGCATTTTGAAAAG GCA [1048]	GGGATAGCACGTAAAAGT GGAA-[1049]
srtA	tanspeptidase;sorta se that anchors surface proteins to the cell wall	91	CTGGTCCTGGATATA CTGGTTCTTT [1128]	GATTAATGACAATCGCTG GTGTG [1129]
sstA	iron transport proteins	52		CTTTGAACAGCACTCGTG CG [1051]
sstB	iron transport protein	53	TATTGCCTTATTTAGA TGTATTGCTTTT [1052]	TCGTAGCTTCAAACACAT TTTCAA [1053]
sstC	iron transport protein	54	AATCAAATGATATTGG AAGATATTAGCA [1054]	TATTCAGTATCTTGTGCTA TTGTCATTG [1055]
sstD	iron transport protein	55	CATGCGGTAACAATT CTGATAAAGA [1056]	AATTTTCGCTTTAGGTGC AGCT [1057]

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stpC	Potential ABC transporter	92	TTAACAATAGAACATT TAACAAAGAAG [1130]	CTCGAAATTAAGAAAGTA ACACC [1131]
tag	DNA-3- methyladenine glycosidase	81	GCATTTGGTACTAAA GATCCAGTCTACT [1108]	AACGAAAATACTGTTACT GGACCTAAAA [1109]
trx	thioredoxin reductase	56	GCTGACTATGAAGGT AAAGCTGACA [1058]	CAGCTAAGTTTTCTTTTG GTTGGA [1059]
tyrA	prephenate dehydrogenase	82		GCTGTCGAATCATTTCTA AAATATACGT [1111]
yhiN	yhiN-protein	57	CAATTGGCTTTCGATT ATTGTTGTA [1060]	AACCAATGATCTAGTGTA AATGTTAAACCT [1061]
	Virulence Factors			
clfA	clumping factor A	3	GCTTCAGTGCTTGTA GGTACGTTAA [952]	TTGATTCACTAATTCCTCC GCAT [953]
clfB	clumping factor B	4	TAATGATACATCTGAT ATTAGTGCAAACAC [954]	TTTAGCATCAGCAGCATT TACTACC [955]
cna	collagen adhesin	85	TCGAGGAATTAACAA AGGTC [1116]	ATCAGGTTTAGTTGGTGG TG [1117]
coa	staphylocoagulase	5	TGTTAGGGATACACA ACATAAAACTGA [956]	GATTITGTTTCAGATTCAC CGTATTT [957]
ebpS	cell surface elastin binding protein	86	GAACCTAGCCATCAA GACAG [1118]	GCATTATTAGAGGCATGT GG [1119]
EDIN	Epidermal cell differentiation inhibitor	113	TATCTTTAGCATTAAG CGTTTATTCAAT [1172]	TTTCTAACTAGATTTTCAT CATACTGGC [1173]
eta	exfoliative toxine A precursor	114	TGCATTTAATTTACCA AAAGAGCTT [1174]	TGGATAGCCTATTAATTC GAGTTTG [1175]
etb	exfoliative toxine B precursor	115	AAGAGCTTTATACACA CATTACGGATAA [1176]	CAAAATATTGAGAATCAT TGAACATTTC [1177]
fbpA	fibrinogen binding protein	88	CTCTTTTTACCTTTGA CGTTGGATT [1122]	GCCAAAATAGTGCTTCAA TATCAGA [1123]
fib	fibrinogen binding protein	89	GCTTTTCTGTGTGCAC TGACAGT [1124]	AGCGAAGGATACGGTCC AAG [1125]
fnbA	fibronectin-binding protein	93		AAACTGCACAACCAGCAA ATATAGA [1133]
fnbB	fibronectin-binding protein	90	CCGCCTTAATTCCTTC TCCAAA [1126]	GCGAGTTGATTTGCCATC GG [1127]
geh	lipase precursor; glycerol ester hydrolase	59		AGGTGCAGTTTTATCATT AGACGG [1065]
hla	alpha-hemolysin	120	ATGATGAAAATGAAA ACACGTATAGTC [1186]	ATTTGAGCTACTTCATTAT CAGGTAGTTG [1187]
hlb	beta-hemolysin	121	TGTTAATAAAGGCACT CCAGAGTTC [1188]	CTTTGATTGGGTAATGAT CTGAAAA [1189]
hld	delta-hemolysin	110	TTTTATCTTAATTAAG GAAGGAGTGATTTC [1166]	TAGTGAATTTGTTCACTG TGTCGATAA [1167]

aacA- aphD	bifunctional aminoglycoside modifying enzyme	843		CTTTTCTTTTGCATAACC TTTTTTC [2633]
	Antibiotic Resistance Determinants			
tst	toxic shock syndrom toxin	138	AAAATTACCTACTCCA ATAGAACTACCTTT [1222]	TTTCTGCTTCTATAGTTTT TATTTCATCA [1223]
sprV8	V8 serine protease gene	137	CAAACA [1220]	CATTGTTGCTGGTTTAAC TACTTCAC [1221]
spa	precursor	94	CTTTAGG [1134]	AGGTTAGCACTTTGACTT GG [1135]
sec	staphylococcal enterotoxin C precursor	129	11 0 1 0 0 1 0 1 1 0 1 - 1 1 -	ATTCCTAGCTTTTATGTCT AGTTCTTGAG [1205]
seb	staphylococcal enterotoxin B precursor	128	ATATATTCTATTAAGG ACACTAAGTTAGGGA AT [1202]	AGTTAGGTAATCTAATTCT TGAGCAGTCA [1203]
sea	staphylococcal enterotoxin A precursor	127	CTGATGTTTTTGATGG	TGCATGTTTTCAGAGTTA ATCGTTT [1201]
sak	staphylokinase	126		GCGCAAAGATCGAAGTCA CTTAT [1199]
nuc	nuclease	71	GCGATTGATGGTGAT ACGGTT [1088]	TTTCGCTTGTGCTTCACT TTT [1089]
NAG	N-acetyl- glucosaminidase; cytotoxin	125		TGCATTTACCCAACCAGT GC [1197]
lukS_N	leucocidin S; C- terminus	123		AATCAAAGCATCTTTGTTA TACTTT [1193]
lukS_C	leucocidin S; C- terminus	124	AGTGTTCAATGGGGA ATAAAAGCTA [1194]	GATCCTTCTAAATAACTAT TGCCATAGTG [1195]
lukF	leucocidin F	122	CATATGGCAGAGATA GTTATCATTCAACT [1190]	GATGTATGAGTTGCTCTT ATGTGATCTTTA [1191]
lip	lipase; glycerol ester hydrolase	68	TTTTAAGTGGTGGAC AAGCACAA [1082]	GATTGTTATTAGCGTTTG AATCTTGAC [1083]
IgGbg	IgG-binding protein	112	GGGTTCTTGCTGTCTT TAAGTGATT [1170]	
hysA	hyaluronate lyase	111	AAACATCAAATCGCT GTGGCT [1168]	GTGAAAGATGCCCTTGAG TGG [1169]
hlgC_C	gamma-hemolysin component C; C- terminus	119		CCAATTGACTTCATATTTC ACAGTGTA [1185]
hlgB	gamma-hemolysin component B	118	ATAGCTTCCACCCAAC ATATGGTAA [1182]	ATTTCACTTTGTGATTTTC CCAATC [1183]
hlgA_N	gamma-hemolysin component A; N- terminus	116	AAGAAAGT [1178]	ATGTTTTGAGTTATAGCT AATCGTT [1179]
hlgA_C	gamma-hemolysin component A; C- terminus	117	ACTGAAGTAGAAAGT CAGAACTCTAAAGGT [1180]	GTGTTTTCCAGTTCACTTC ATATTTAACT [1181]

aadD	aminoglycoside acetyl transferase; kanamycin resistance	837	AAGCAGAGTTCAGCC ATGAATG [2620]	CAGATGCGATGATGCAGA CC [2621]
aphA3	3' 5'-aminoglycoside acetyltransferase; kanamycin resistance	845	GAAAACC [2636]	CCAGTTTTCGCAATCCAC ATC [2637]
blaI	regulator protein	814	AGCAAGTTGAAATAT CTATGGCTGA [2574]	TCATTTAAAATGTCTCGCA ATTCTT [2575]
blaR	beta lactamase repressor	790		GCATTTTTCCCAGATGGC TT [2527]
blaZ	beta-lactamase	827	GATAAGAGATTTGCC TATGCTTCAA [2600]	TGCTTAATTTTCCATTTGC GAT [2601]
cadA	Probable cadmium- transporting ATPase (Cadmium efflux ATPase)	897	TTGGATAGTTCAACAA AAACATTAACA [2740]	
cadC	Cadmium efflux system accessory protein homolog	908	TAGCAACCTCCCTTTG ATAC [2762]	ACAAAAGATATGTGTGAA GTTACC [2763]
cat	chloramphenicol acetyltransferase	862	CCTTCTTTGATTTATG CAATTATGG [2670]	GAAGCATGGTAACCATCA CATACA [2671]
dfrA	S1 dihydrofolate reductase; trimethoprim resistance	859	ATGACATTATCAATAA	AACATGACCAGATAACTC TITAATTTCAT [2665]
ermA	rRNA methylase	852	TAGCTATCTTATCGTT GAGAAGGGAT [2650]	AAAGAAATTGTTCCTTCG ATAGTTTATT [2651]
ermB	adenine methylase	851	AACCGATACCGTTTAC GAAATTG [2648]	CGCTTGTAGAATCCTTCT TCAACA [2649]
ermC	adenine methylase	846	AACACAGTCAAAACTT TATTACTTCAAAAC [2638]	TTGCATAATTTATGGTCTA TTTCAATG [2639]
femA	factor essential for methicillin resistance	801	TAGGATTTGAACATAC TGGATTCCA [2548]	AAAGGCACTAACACACGG TCTTT [2549]
femD	putative factor essential for methicillin resistance	16	TCAGGTGAAATGTTA GAATCAGCA [978]	TAAGTCACCAAATAAGAA TGGCG [979]
fmhA	similar to Staphylococcus aureus FemA and FemB proteins	825	GTTAACGATTGATGA AACGCAAA [2596]	TGCACCATCTTGTTCAATT TGTT [2597]
fmhB	essential for addition of glycine 1 to peptidoglycan precursor	818	GAGTTATTAAATAGTT TTGAACGCCG [2582]	TTCAGGATGTTCCTTTTCT AAAGCT [2583]
linA	lincosaminide	850	GATATAGGATACAAA ATAGAAGTTGATTGG [2646]	GGTCTTTTTCTGTTAATTC ATAACCG [2647]

mecA	penicillin binding protein 2'	802		CTAATAGATGTGAAGTCG CTTTTCCT [2551]
mecI	mecI protein	812	TAATAAAACGTATGAA ATATCATCTGCA [2570]	TTTCATCTTGTGATAGATC TTCTTTTTC [2571]
mecR	mecI protein	798	TTTAAAGAATGGAAC CAAGATCAAA [2542]	TCGCCTTTTAAATGTGTA GCAAA [2543]
mreA	ABC transporter	907	GCAGTATTAGTACTTG ATGAACCAACG [2760]	GACAAAACGTACAGGATG TCCATAA [2761]
mreB	ABC transporter	36	ATGAGGTACTCTTTAA TTAGTGGTATCTTGA [1018]	ATCAGCTAATGAAATGAA GATTGCA [1019]
mreR	ABC transporter	37	GAAAATACAGAACTT GATGGTGAAATG [1020]	GCAAGACTCACATACACC ATAAACTTC [1021]
msrA	methionine sulfoxide reductase	854	TCATAAGCTGACAGA TTTTCGATCC [2654]	CTTTTAGATGAACCTACA AATCACTTGG [2655]
norA	quinolone resistance protein	904	TTAGCTTTCATAATGT CAGTTGTATTGA [2754]	ACAGTGTTTCAAATGCCG ATAAA [2755]
pbpF	penicillin-binding protein Pbp2b	42		CTATCCCAATCCATAGAC GTGTTAA [1031]
qacA	quaternary ammo- nium compound resistance protein	885		GCCCACTACAGATTCTTC AGCTAC [2717]
spc	adenyltransferase AAD9	844	ATATCAGGAAAGATT GGAAATACGG [2634]	AAAGAGGTATAGCCCATT CTGCA [2635]

In order to obtain a high specificity level, each selected gene was compared to all other gene sequences available in the NCBI database using the BLAST algorithm. From that comparison, regions (ranging from 104 to 1434 bp) devoid of apparent homology with genes of other bacterial species and Homo sapiens were defined and amplified by PCR using specifically designed primers (see Tab. 6). A mixture of the total DNA from three different S. aureus reference strains and 100 clinical isolates was used as template for amplification of S. aureus gene segments, increasing therefore the chances to amplify more seldom occurring virulence and antibiotic resistance genes. PCR products were cloned into the plasmid pCR 2.1-Topo Vector (Invitrogen, Karlruhe, Germany) which were used to transform competent Escherichia coli (XL-1-Blue) cells using the Calcium Chloride protocol (Seidman, C.E. et al., in: Ausubel, F.M. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (2000)). Recombinant plasmids containing selected gene segments were screened by restriction analysis and verified by sequencing. The plasmid library constructed was used for re-amplification and production of the bulk DNA (10 µg at a concentration of 1 µM) from each clone necessary for printing the

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microchips. A Microgrid II spotter (BioRobotics, Cambridge, UK) and CMT-GAPS<sup>™</sup> coated glass slides (Corning Incorporated, Corning, USA) were used. The complete array of 140 segments of genes was spotted in 3 replicates per slide.

### C) DNA purification

## 5 <u>a) Sample preparation</u>

<u>Bacterial cultures:</u> Overnight cultures (5 ml) were harvested at 2,560g for 10 minutes. After discarding the supernatant the pellet was washed in 1ml TE (10 mM Tris-HCl, pH 7.5 - 1 mM EDTA) and recovered by centrifugation at 17,900 g for 2 min.

Blood cultures: One ml of blood culture was mixed with 1 ml 0.1% Triton®-X-100 and kept at room temperature for 5 min in order to disrupt blood human cells and resolve bacterial clumps. Bacterial cells were then harvested at 17,900 g for 10 min. Pellets were washed in 1 ml TE and recovered as described above.

## b) Purification of DNA

15 Pellets of harvested cells were resuspended in 500 µl lysis buffer (20 mM Tris-HCl, pH 8.0 - 2 mM EDTA, pH 8.0 - 1.2% Triton<sup>®</sup>-X-100). To promote bacterial lysis, lysozyme and lysostaphin (Sigma, Taufkirchen, Germany) were added to reach a final concentration of 0.8 mg/ml and 0.2 mg/ml respectively. To lyse Gram negative bacterial cells, only lysozyme in the indicated concentration was used. Samples were then incubated for one hour at 37°C. After treatment with Proteinase 20 K (1 mg/ml) (Sigma, Taufkirchen, Germany) for 5 hours at 55°C under mild agitation, the samples were heated at 65°C for 30 min to inactivate Proteinase K and then cooled down to 37°C. Finally, a RNAse A treatment (0.2 mg/ml) was 37°C. carried out for hour at Α pre-treatment with 1 (Cethyltrimethylammonium bromide) was performed in order to release DNA from 25 polysaccharide DNA complexes (Murray, M.G. and Thopson, W.F., Nucl. Acid Res. 8:4321-4325 (1980)). Salt concentration was adjusted to 0.7 M by adding 5 M NaCl. After thoroughly mixing, a 10% CTAB-0.7M NaCl solution was added to adjust the CTAB concentration to 1%.

The mixture was subsequently incubated under rotation for 20 min at 65°C and then extracted with one volume of chloroform/isoamyl alcohol (24:1). The samples were spun in a microcentrifuge (17,900 g) at room temperature. The aqueous phase was extracted once with chloroform/isoamyl alcohol (24:1), once with phenol/chloroform/isoamyl alcohol (25:24:1) and finally with chloroform/isoamyl alcohol (25:24:1). Genomic DNA in the aqueous phase was sonified (3 x 10 s at 12% amplitude with 20 s breaks between pulses) in a Digital Sonifier (Branson, Schwaebisch Gmuend, Germany) to obtain fragments of around 1 kb, then precipitated with one volume of isopropanol and pelleted by centrifugation for 30 min at 4°C in a microcentrifuge at 17,900 g. The pellets were washed in 70% ethanol and resuspended in 50-100  $\mu$ l TE (10 mM Tris-HCl, pH 7.5 - 1 mM EDTA). This DNA preparation was used when a high yield (hundreds of  $\mu$ g) was necessary, for example to prepare samples for several hybridisations experiments.

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A second protocol using DNeasy Tissue Kit (QIAGEN, Hilden, Germany) adapted to bacterial cells and allowing DNA preparation in two hours, was also used when fast preparation was the priority. The abbreviations below pertain to the manufacturer's abbreviations for buffers used in the kit. The bacterial pellet was resuspended in 1 ml ddH<sub>2</sub>O and the cell suspension frozen in liquid N<sub>2</sub> for 1 minute and then placed in a 60° C thermo-block for 2 minutes. Such a treatment was repeated once and bacteria were centrifuged again for 5 minutes at 14,000g. The resulting pellet was resuspended in 180 µl lysis buffer (20 mM Tris-HCl, pH 8.0 - 2 mM EDTA, pH 8.0 -1.2% Triton-X-100). Specifically for S. aureus DNA preparation, lysostaphin (0.2mg/ml) was added and incubated 1 hour at 37°C. After, 200 µl of buffer AL (for gram positive bacteria) or buffer ATL (for gram negative) and 25 µl of the Proteinase K solution delivered with the kit were added and incubated at 70°C for 30 minutes. 200 µl of 100% ethanol were added and the suspension transferred to a DNeasy Mini Column placed into a collection tube. The column was centrifuged at 6,000 g for 1 minute, washed first with 500 µl of buffer AW1, centrifuged at 6,000 g for 1 minute, washed then with 500 µl of buffer AW2, and centrifuged at 14,000 g for 3 minutes. The column was then placed in a 1.5 ml tube and centrifuged once more at 14,000 g for 1 minute. DNA was eluted with 130 µl of buffer AE. After one minute the column was centrifuged at 6,000g for 1 minute. The eluate was reloaded in the column and centrifuged again under the same conditions in order to increase the DNA yield.

## D) DNA labelling

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Different amounts of DNA (5 ng to 5  $\mu$ g) were labelled with 3  $\mu$ l either of Cy5-dCTP or Cy3-dCTP (Amersham Pharmacia Biotech Europe, Freiburg, Germany) by random priming (1 x random primer/Klenow reaction buffer) using Klenow Polymerase (50units) (both from BioPrime DNA labelling Kit, Invitrogen, Karlsruhe, Germany) in the presence of 0.12 mM dATP's, dGTP's and dTTP's and 0.06 mM dCTP's, in a total volume of 50  $\mu$ l. After 2 hours incubation at 37°C, the reaction was interrupted by adding 5  $\mu$ l of 0.5 M EDTA and the probe purified either by MiniElute PCR or QIAquick Purification Kits (QIAGEN, Hilden, Germany), depending on the amount of labelled DNA applying two wash and two elution steps.

## E) Hybridisation and detection procedure

All experiments described in the present example represent co-hybridisation of two different DNA samples labelled respectively with Cy3 and Cy5. Cy3 and Cy5 belong to the cyanine family of fluorophores and were used as reporter molecules. The photochemical properties of the two CyDye fluors were as follows: Absorption maximum at 550 nm and emission maximum at 570 nm for Cy3 and for Cy5 at 649 nm and 670 nm, respectively.

20 After purification, Cy3 and Cy5 labelled DNA were pooled and 10 µg of Salmon Sperm DNA and 50 µg of polyA DNA were added. The mixture was frozen in liquid nitrogen and lyophilized in the dark. DNA microchips were automatically hybridised in a GeneTac Hybridisation Station (Genomic Solutions, Harvard, USA) following the Corning protocol.

Shortly, 110 μl of pre-hybridisation buffer (25% Formamide, 5x SSC, 0.1% SDS, 10 mg/ml BSA) were added to each slide and incubated for one hour at 42°C. Lyophilized samples were resuspended in 110μl of hybridisation buffer (25% Formamide, 5x SSC, 0.1% SDS), denatured for 3 minutes at 90°C, added to the slides, and incubated 4 hours at 42°C. After several washing steps using successively 2 x SSC/0.1% SDS, 0.1 x SSC/0.1% SDS, and 0.1 x SSC, slides were

dried by a 2 min centrifugation step (1000 g) and read in a Scan Array 5000 (Perkin Elmer, Boston, USA) using emission filters for Cy3 and Cy5 in two separate channels. Fluorescence intensities as hybridisation indicators were then analyzed by the software ImaGene (BioBiscovery, Marina Del Rey, USA). Spots were found and segmented in order to select areas of recognizable signals for analysis. Intensity of fluorescence of each spot was measured, signal to local background ratios were calculated, spot morphology and deviation from expected spot position were considered. Cut off values for those parameters were empirically determined in pilot experiments and used to tag spots either as positive or as negative.

## 10 F) Validation of the detection system

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The experimental approach adopted in present example required dual-dye hybridisations. It was therefore necessary to verify at first whether DNA samples from the same source, labelled with one or the other fluorochrome, would produce the same hybridisation pattern. Co-hybridisation experiments, combining two identical samples of 2  $\mu$ g of *S. aureus* DNA, produced strictly similar hybridisation results whatever fluorochrome was used for labelling (Fig. 2A). For better presentation gray scale images from scanning were converted in false-colour, where green and red colour represent intensity of Cy3 and Cy5 fluorochromes respectively. All spots showed double-hybridisation - yellow colour meaning the overlay between green (here assigned to Cy3 labelled DNA) and red signals (Cy5 labelled DNA). Signal intensities from both channels strongly correlated ( $r^2$ =0,97) (Fig. 2B).

### G) Sensitivity of detection

S. aureus DNA samples in decreasing amounts (from 2 µg to 5 ng) were labelled and hybridised in order to determine the minimum amount of DNA producing the expected hybridisation pattern for a certain strain. Such expected patterns were defined as those produced by the hybridisation of 2 µg of DNA. From 2 µg to 50 ng no significant differences in the hybridisation pattern were observed with no false negative spots. Detection of 20 ng DNA was still satisfying with only 5% of false negative and false positive. However, 5 ng of labelled DNA yielded weak signals with almost 95% of false negative spots (data not shown). The limit of sensitivity of the S. aureus microarray was then considered as being 20 ng DNA which

corresponds approximately to 7 x  $10^6$  *S. aureus* CFU (*S. aureus* genome 2.5 x  $10^6$  bp. 2.8 fg DNA per cell).

#### H) Specificity of detection

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The specificity of the *S. aureus* microchip was demonstrated by six independently performed co-hybridisation experiments. Visual examination of pictures showing results of co-hybridisation of *S. aureus* DNA with *Pseudomonas aeruginosa* or *Escherichia coli* DNA revealed no cross-hybridisation between *S. aureus* selected gene segments and DNA probes from those Gram negative bacteria (data not shown). Transcribing these data in a bar code showing positive or negative spots (Fig. 3A and B) confirmed that only the *S. aureus* DNA sample hybridised with spotted probes.

The specificity of the microarray could be demonstrated even below the genus level. As shown in Fig. 4, some spotted *S. aureus* probes cross-hybridised with *S. epidermidis* and *S. saprophyticus* DNA samples. This is not surprising as these species are phylogenetically closely related. However, genes coding for *S. aureus* specific proteins as nuclease (*nuc*), clumping factors A and B (*clfA* and B), protein A (*spa*), V8 serine protease (*sprV8*) and alpha and beta hemolysins (*hla* and *hlb*) exclusively hybridised with *S. aureus* DNA. The presence/absence of such genes allowed unambiguous discrimination between *S. aureus* and CoNS.

#### 20 <u>I) S. aureus strain profiling</u>

The principle of the *S. aureus* microarray was tested as a tool for strain profiling. A distinctive hybridisation pattern could be established for reference strains and 10 selected clinical isolates. For instance when DNA from clinical isolates T100 and T103 were labelled with Cy5 and Cy3, respectively, and co-hybridised, both isolates were identified as *S. aureus*, since both contained species-specific genes as e.g. clumping factor A and B (Fig. 5A).

Moreover, both strains are methicillin resistant (*mecA* positive), but only T100 contained the beta-lactamase gene. The hybridisation of T103 DNA reveals the presence of *ermA*, *ermB* and *aacA* genes indicating that the strain is resistant to erythromycin and aminoglycosides.

Apparently, T103 harbors the genes encoding enterotoxines A (eta) and B (etb) while in T100 the gene encoding enterotoxin C (etc) is present. The presence or absence of these genes was confirmed by PCR assays (Fig. 5B) and the antibiotic resistance was verified by classical antibiograms (Sahm, D. & Washington, J. A. (1991). Antibacterial susceptibility tests: dilution methods. In: Manual of Clinical Microbiology (Balows, A., Ed.), pp. 1105–16. American Society for Microbiology, Washington DC, USA) (data not shown).

## J) Detection of S. aureus in spiked positive BACTEC® cultures

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One possible application of the *S. aureus* microarray is to detect the bacterium growing in blood culture, i.e. after the BACTEC® signals bacterial growth. Blood culture bottles were spiked with 100 CFU of *S. aureus*. After the automated culturing system indicated bacterial growth, 1 ml was withdrawn for DNA extraction.

As shown in Fig. 6A, DNA samples prepared from sterile blood culture show no crosshybridisation with spotted *S. aureus* probes. A 2 µg DNA sample derived from blood culture containing *S. aureus* cells revealed a hybridisation pattern almost completely identical to a DNA sample isolated from an overnight LB culture inoculated with a *S. aureus* colony (Fig. 6B).

These data underscore the high sensitivity and specificity of the detection system since blood culture DNA comprises a mixture of human and bacterial DNA. Cohybridisation between DNA from blood culture positive for *S. aureus* and CoNS DNA also allowed clear identification since only the *S. aureus* probe hybridised to *S. aureus* species-specific genes (data not shown).

# K) Detection of *S. aureus* in positive BACTEC® cultures inoculated with clinical specimens

Co-hybridisation with DNA from clinical blood cultures positive for *S.aureus* and CoNS (*Staphylococus epidermidis*), *Streptococcus mitis*, *E. coli* and *Klebsiella oxytoca* allowed clear species identification since the *S.aureus* probes hybridised to *S.aureus* species-specific genes only. *Staphylococcus epidermidis* positive blood culture DNA hybridised to staphylococcal metabolic genes and to some antibiotic

resistance determinant genes only. No cross-hybridisation was detected between DNA from the two gram-negative strains and the *Streptococcus* strain and *S. aureus* spotted gene probes (data not shown).

# Example 2.1: Materials and Methods

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Reference strains, clinical isolates and culture conditions: Bacterial reference strains were obtained from the American Type Culture Collection (ATCC, Manassas, Va.), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), the Collection Institute Pasteur (CIP, Paris, France) or the network on antimicrobial resistance in *Staphylococcus aureus* (NARSA, Herndon, Virginia). *Klebsiella pneumoniae* serotype O3 and serotype O8 were provided by E.M. Nielsen (Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark). Clinical isolates were obtained from the inventors' clinical routine microbiology laboratory.

The following bacteria and fungi were used for evaluation of the specificity of the microarray: Acinetobacter baumannii (DSM 30008, 1 clinical isolate), Pseudomonas aeruginosa (ATCC27853), Escherichia coli (ATCC 25922, CIP 105893, 81.88, 74.14 and 3 clinical isolates), Klebsiella oxytoca (DSM 4798, 1 clinical isolate), Klebsiella pneumoniae (DSM 681, serotype O3 strain 390 and serotype O8 strain 889), Proteus mirabilis (DSM 788, 2 clinical isolates), Proteus vulgaris (DSM 2140), Candida albicans (ATCC 10231), Enterococcus casseliflavus (clinical isolate), Enterococcus faecalis (ATCC 29212, 1 clinical isolate), Enterococcus faecium (clinical isolate), Enterococcus gallinarum (clinical isolate), Streptococcus agalactiae (DSM 2134), Streptococcus angiosus (DSM 20563), Streptococcus bovis (DSM 20480), Streptococcus dysgalactiae (DSM 20662), Streptococcus gordonii (DSM 6777), Streptococcus mutans (DSM 20523), Streptococcus pneumoniae (ATCC 49619), Streptococcus pyogenes (DSM 11723), Staphylococcus aureus (ATCC 29213, NRS123 alias MW2, 2 clinical isolates), Staphylococcus epidermidis (ATCC 1 clinical isolates), Staphylococcus haemolyticus (DSM 12228, Staphylococcus hominis (DSM 20228), Staphylococcus lugdunensis (DSM 4804), Staphylococcus saprophyticus (ATCC 14953) and Staphylococcus warneri (DSM 20316).

Bacterial and fungal reference strains and clinical isolates were grown over night at  $37\,^{\circ}\text{C}$  with constant shaking in 5 ml Luria-Bertani (LB) broth or tryptic soy broth

(TSB, 30 g/l, Merck) containing 3 g/l yeast extract. Enterococci and streptococci were grown in 10 ml TSB plus yeast without agitation under 5% CO<sub>2</sub>. Overnight cultures were harvested at 2,560 g for 10 min. After discarding the supernatant the pellet was washed in 1 ml TE (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA) and recovered by centrifugation at 17,900 g for 10 min. Cell pellets were used for DNA preparation.

## Example 2.2: DNA preparation

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For microarray hybridization experiments, DNA was prepared from the strains listed in Example 2.1.

Total cellular DNA was extracted and purified by using the Bacterial Genomic DNS Purification Kit (Edge BioSystems, Gaithersburg, USA). Cell pellets were resuspended in 200 µl lysis buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl and 10 mM EDTA, pH 8.0) and lysozyme (Sigma, Taufkirchen, Germany) was added to reach a final concentration of 7.5 mg/ml. In additon, lysostaphin (Sigma) was added to a final concentration of 0.2 mg/ml to promote Staphylococcal lysis or mutanolysin (0.5 U/µl; Sigma) was added to lyse Streptococci and Enterococci. After incubation at 37°C for one hour, 400 µl Sphaeroblast buffer were added and DNA was extracted following the instructions of the supplier.

20 Candida albicans DNA was extracted using the MasterPure Yeast DNA purification kit (Epicentre Biotechnologies, Madison USA) following the instructions of the manufacturer.

Concentration, purity and size of the purified DNA preparations were determined by UV-spectrophotometry (lambda 40, PerkinElmer, Boston USA) and 1% agarose gel electrophoresis.

#### Example 2.3: DNA labelling

Prior to labelling, high molecular weight DNA ( $\geq$ 12 kb) was fragmented by sonication for 30 sec at an amplitude of 80% (energy input 1500 kJ) using an ultrasonic homogenizer (Sonoplus HD 3080, Bandelin, Berlin, Germany) equipped with a BR30 booster cup for high-intensive irradiation of small and sensitive sample volumes. The size of the fragmented DNA (500-8000 bp) was checked by 1.5% agarose gel electrophoresis. Different amounts of DNA (1 to 5  $\mu$ g) were then labeled with 3  $\mu$ l either of Cy5-dCTP or Cy3-dCTP (Amersham Pharmacia Biotech

Europe, Freiburg, Germany) by random priming (1 x random primer/Klenow reaction buffer) using Klenow Polymerase (50 units) (both from BioPrime DNA labeling Kit, Invitrogen, Karlsruhe, Germany) in the presence of 0.12 mM dATP's, dGTP's and dTTP's and 0.06 mM dCTP's, in a total volume of 50 µl. Prior to labelling, each target DNA was spiked with three gene segments (1 µl each, 30 ng/µl) amplified by PCR from selected recombinant plasmids to serve as internal positive controls. After 2 hours incubation at 37°C, the reaction was interrupted by adding 5 µl of 0.5 M EDTA and unbound label was removed using the QIAquick Purification Kit (QIAGEN, Hilden, Germany). The purified labelled DNA was eluted in 80 µl TE and the relative labelling efficiency of a reaction was evaluated by calculating the approximate ratio of bases to dye molecules (acceptable labelling ratios for nucleic acid were ≤60). This ratio and the amount of recovered labelled DNA was determined by measuring the absorbance of the nucleic acids at 260 nm and the absorbance of the dye at its absorbance maximum using a lambda40 UVspectrophotometer (PerkinElmer) and plastic disposable cuvettes for the range from 220 nm to 1,600 nm (UVette; Eppendorf, Hamburg, Germany).

#### Example 2.4: Microarray construction

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Cloned PCR-products were used to generate probes for the DNA microarray. All together 930 gene segments ("probes") were represented on the microarray (Tab. 7). They comprised probes for virulence genes, species specific metabolic and structural genes from *Candida albicans* (86), *Acinetobacter baumannii* (21), *Enterobacter cloacae* (11), *Escherichia coli* (31), *Enterococcus faecalis* (69), *E. faecium* (23), *Klebsiella oxytoca* (21), *K. pneumoniae* (50), *P. aeruginosa* (53), *Proteus mirabilis* (70), *P. vulgaris* (9), *Stenotrophomonas maltophilia* (13), *Streptococcus agalactiae* (38), *S. dysgalactiae* (1), *S. pneumoniae* (83), *S. pyogenes* (42), *S. viridans* (19, including probes for *S. mutans* and *S. bovis*), Streptococci (2), *Staphylococcus aureus* (69), *S. epidermidis* (35), *S. haemolyticus* (7), *S. hominis* (1), *S. lugdunensis* (6), *S. saprophyticus* (2) and *S. warneri* (7), as well as for bacterial antibiotic resistant determinants (131), and positive and negative controls (29).

<u>Tab. 7:</u> Gene probes on array of example 2.

n Probe Name S	eqID
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n	Probe Name	SeqID
1	16SKpn_1_1	934
2	16SrRNAPrmi_1_1	940
3	16SRNAEf_1_1	936
4		933
5	16SShaemolyt_1_1	938
	16SShominis_1_1	937
$\overline{}$	16SStrepagalactiae_1_1	930
	16SPa 1 1	926
	16SSa 1 1	942
	16SSa_3_1	935
	16SStrepneu_1_1	929
	16SStrepyog_1_1	928
	16SKlox_1_1	943
_	16SrRNAPrvu1_1_1	941
_		
	16SEfaecium_1_1 16SEfaecium 2 1	931 932
	23SEfaecium_1_1	939
	23SEfaecium_2_1	927
	ARHGDIA(hu)_1_1	923
Company of the last	b-Act(hu)_1_1	922
_	GAPD(hu)_1_1	921
	LDHA(hu)_1_1	920
	PGK1(hu)_1_1	924
	rbcL_1_1	919
_	rbcL_1_2	925
	aac(6p)-lb7_1_1	2867
	aacA-aphD_1_1	843
	aacA4ENCL_1_1	2864
	aacC2_1_1	833
	aadB_1_2	836
	aadD_1_1	837
32	adeA-ACIBA_1_1	2866
33	adeB-ACIBA_1_1	2868
	adeC-ACIBA_1_1	2869
35	AdeR-ACIBA_1_1	2865
36	AdeS-ACIBA_1_1	2870
37	aph-A3_1_1	840
38	strA_1_1	839
39	strB_1_1	834
40	aacA_aphDStwar_1_1	831
41	aacA4_1_1	842
42	aacA4_1_2	838
43	aacC1_1_1	841
	aacC1_1_2	832
45	aadA_1_1	835
	aphA3_1_1	845
	<del></del>	

47 ampC-ENCL_1_1 2874 48 ampC_1_1 789 49 ampR_1_1 2873 50 blaA_1_1 823 51 blaB_1_1 788 52 blalShaemolyt_1_1 803 53 blaL1_1_1 2875 54 blaL2_1_1 2871 55 blaMIR-3_1_1 2872 56 blaOXA-1_1_1 828 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 793 72 pbp2x_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 809 75 pbp5Efaecium_1_1 809 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 80 blaIMP-7_1_2 879 81 blaIMP-7_1_2 797 82 blaOXA-32_1_1 795 84 blaOXA-32_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829	П	Probe Name	SeqID
48 ampC_1_1 789 49 ampR_1_1 2873 50 blaA_1_1 823 51 blaB_1_1 788 52 blalShaemolyt_1_1 803 53 blaL1_1_1 2875 54 blaL2_1_1 2871 55 blaMIR-3_1_1 2872 56 blaOXA-1_1_1 828 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 793 72 pbp2x_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 75 pbp5Efaecium_1_1 809 75 pbp5Efaecium_1_1 809 75 pbp5Efaecium_1_1 822 80 blaIMP-7_1_2 809 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 822 81 blaOXA-2_1_1 795 82 blaOXA-2_1_1 795 83 blaOXA-2_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			<u> </u>
49 ampR_1_1			<del></del>
50 blaA_1_1	_		<del></del>
51 blaB_1_1       788         52 blalShaemolyt_1_1       803         53 blaL1_1_1       2875         54 blaL2_1_1       2871         55 blaMIR-3_1_1       2872         56 blaOXA-1_1_1       816         57 blaOXY-KLOX_1_1       816         58 blaSHV-1_1_1       794         59 blaTEM-106_1_1       815         60 blavim_1_1       804         61 blaZ_1_1       827         62 cumA_1_1       819         63 femA_1_1       801         64 femBShaemolyt_1_1       820         65 fmhA_1_1       825         66 fmhB_1_1       818         67 ftsWEF_1_1       817         68 mecA_1_1       802         69 mecISepid_1_1       786         70 pbp1a_1_1       813         71 pbp2aStrpneu_1_1       793         72 pbp2x_1_1       807         73 pbp3Saureuc_1_1       808         74 pbp4_1_1       809         75 pbp5Efaecium_1_1       810         76 pbpC_1_1       811         77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_2       797<			
52 blalShaemolyt_1_1 803 53 blaL1_1_1 2875 54 blaL2_1_1 2871 55 blaMIR-3_1_1 2872 56 blaOXA-1_1_1 816 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 783 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 809 75 pbp5Efaecium_1_1 820 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 799 86 blaPER-1_1_1 830 88 blaPShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829 91 mecR1Sepid_1_1 829	$\vdash$		<u> </u>
53 blaL1_1_1 2875 54 blaL2_1_1 2871 55 blaMIR-3_1_1 2872 56 blaOXA-1_1_1 828 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1 1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 80 blaIMP-7_1_2 797 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 799 86 blaPER-1_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 829 91 mecR1Sepid_1_1 829			
54 blaL2_1_1 2872 55 blaMIR-3_1_1 828 57 blaOXA-1_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 819 67 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp1a_1_1 807 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 80 blaIMP-7_1_2 797 81 blaOXA-10_1_2 787 83 blaOXA-2_1_1 799 86 blaPER-1_1_1 820 87 blaPrmi_1_1 820 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
55 blaMIR-3_1_1 828 56 blaOXA-1_1_1 828 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp1a_1_1 803 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 80 blaIMP-7_1_2 797 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 799 86 blaPER-1_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 796 88 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
56 blaOXA-1_1_1 828 57 blaOXY-KLOX_1_1 816 58 blaSHV-1_1_1 794 59 blaTEM-106_1_1 815 60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_2 797 81 blaOXA-2_1_1 795 84 blaOXA-2_1_1 799 85 blaOXA-10_1_2 787 87 blaPFmi_1_1 799 86 blaPER-1_1_1 829 91 mecR1Sepid_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
57 blaOXY-KLOX_1_1	$\vdash$		<del></del>
58 blaSHV-1_1_1			
59 blaTEM-106_1_1 815 60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_2 797 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 820 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829 91 mecR1Sepid_1_1 829			<del></del>
60 blavim_1_1 804 61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_2 797 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 821 87 blaPrmi_1_1 829 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
61 blaZ_1_1 827 62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_2 797 81 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 820 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			
62 cumA_1_1 819 63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 824 80 blaIMP-7_1_2 797 81 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 820 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
63 femA_1_1 801 64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 824 80 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 820 87 blaPFR-1_1_1 820 88 blaPER-1_1_1 821 87 blaPrmi_1_1 820 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 829			
64 femBShaemolyt_1_1 820 65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 786 70 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 799 86 blaPER-1_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 829 91 mecR1Sepid_1_1 829			-
65 fmhA_1_1 825 66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 820 86 blaPER-1_1_1 821 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			
66 fmhB_1_1 818 67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 821 87 blaPER-1_1_1 830 88 blaPER-1_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826	_		
67 ftsWEF_1_1 817 68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 820 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			<del> </del>
68 mecA_1_1 802 69 meclSepid_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 809 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 820 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			
69 meclSepid_1_1 786 70 pbp1a_1_1 813 71 pbp2aStrpneu_1_1 793 72 pbp2x_1_1 807 73 pbp3Saureuc_1_1 808 74 pbp4_1_1 809 75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 821 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			<u> </u>
70       pbp1a_1_1       813         71       pbp2aStrpneu_1_1       793         72       pbp2x_1_1       807         73       pbp3Saureuc_1_1       808         74       pbp4_1_1       809         75       pbp5Efaecium_1_1       810         76       pbpC_1_1       811         77       psrb_1_1       824         78       bla-CTX-M-22_1_1       792         79       bla_FOX-3_1_1       822         80       blaIMP-7_1_1       785         81       blaIMP-7_1_2       797         82       blaOXA-10_1_2       787         83       blaOXA-2_1_1       791         84       blaOXA-32_1_1       791         85       blaOXY_1_1       799         86       blaPER-1_1_1       820         87       blaPimi_1_1       830         88       blaRShaemolyt_1_1       796         89       dacCStrpyog_1_1       809         90       fox-6_1_1       829         91       mecR1Sepid_1_1       826	$\vdash$		
71 pbp2aStrpneu_1_1         793           72 pbp2x_1_1         807           73 pbp3Saureuc_1_1         808           74 pbp4_1_1         809           75 pbp5Efaecium_1_1         810           76 pbpC_1_1         811           77 psrb_1_1         824           78 bla-CTX-M-22_1_1         792           79 bla_FOX-3_1_1         822           80 blaIMP-7_1_1         785           81 blaIMP-7_1_2         797           82 blaOXA-10_1_2         787           83 blaOXA-2_1_1         791           84 blaOXA-32_1_1         791           85 blaOXY_1_1         799           86 blaPER-1_1_1         821           87 blaPrmi_1_1         830           88 blaRShaemolyt_1_1         796           89 dacCStrpyog_1_1         800           90 fox-6_1_1         829           91 mecR1Sepid_1_1         826			
72 pbp2x_1_1       807         73 pbp3Saureuc_1_1       808         74 pbp4_1_1       809         75 pbp5Efaecium_1_1       810         76 pbpC_1_1       811         77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       791         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
73 pbp3Saureuc_1_1       808         74 pbp4_1_1       809         75 pbp5Efaecium_1_1       810         76 pbpC_1_1       811         77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       795         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
74 pbp4_1_1       809         75 pbp5Efaecium_1_1       810         76 pbpC_1_1       811         77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       791         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
75 pbp5Efaecium_1_1 810 76 pbpC_1_1 811 77 psrb_1_1 824 78 bla-CTX-M-22_1_1 792 79 bla_FOX-3_1_1 822 80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 821 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			
76 pbpC_1_1       811         77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       795         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
77 psrb_1_1       824         78 bla-CTX-M-22_1_1       792         79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       795         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
78       bla-CTX-M-22_1_1       792         79       bla_FOX-3_1_1       822         80       blaIMP-7_1_1       785         81       blaIMP-7_1_2       797         82       blaOXA-10_1_2       787         83       blaOXA-2_1_1       795         84       blaOXA-32_1_1       791         85       blaOXY_1_1       799         86       blaPER-1_1_1       821         87       blaPrmi_1_1       830         88       blaRShaemolyt_1_1       796         89       dacCStrpyog_1_1       800         90       fox-6_1_1       829         91       mecR1Sepid_1_1       826			<del>                                     </del>
79 bla_FOX-3_1_1       822         80 blaIMP-7_1_1       785         81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       795         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			<u> </u>
80 blaIMP-7_1_1 785 81 blaIMP-7_1_2 797 82 blaOXA-10_1_2 787 83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 821 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826			822
81 blaIMP-7_1_2       797         82 blaOXA-10_1_2       787         83 blaOXA-2_1_1       795         84 blaOXA-32_1_1       791         85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			785
82 blaOXA-10_1_2     787       83 blaOXA-2_1_1     795       84 blaOXA-32_1_1     791       85 blaOXY_1_1     799       86 blaPER-1_1_1     821       87 blaPrmi_1_1     830       88 blaRShaemolyt_1_1     796       89 dacCStrpyog_1_1     800       90 fox-6_1_1     829       91 mecR1Sepid_1_1     826			797
83 blaOXA-2_1_1 795 84 blaOXA-32_1_1 791 85 blaOXY_1_1 799 86 blaPER-1_1_1 821 87 blaPrmi_1_1 830 88 blaRShaemolyt_1_1 796 89 dacCStrpyog_1_1 800 90 fox-6_1_1 829 91 mecR1Sepid_1_1 826	82		787
84 blaOXA-32_1_1     791       85 blaOXY_1_1     799       86 blaPER-1_1_1     821       87 blaPrmi_1_1     830       88 blaRShaemolyt_1_1     796       89 dacCStrpyog_1_1     800       90 fox-6_1_1     829       91 mecR1Sepid_1_1     826			795
85 blaOXY_1_1       799         86 blaPER-1_1_1       821         87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
86       blaPER-1_1_1       821         87       blaPrmi_1_1       830         88       blaRShaemolyt_1_1       796         89       dacCStrpyog_1_1       800         90       fox-6_1_1       829         91       mecR1Sepid_1_1       826	-	<del></del>	799
87 blaPrmi_1_1       830         88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			
88 blaRShaemolyt_1_1       796         89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			830
89 dacCStrpyog_1_1       800         90 fox-6_1_1       829         91 mecR1Sepid_1_1       826			796
90 fox-6_1_1     829       91 mecR1Sepid_1_1     826	89		800
91 mecR1Sepid_1_1 826	$\overline{}$		829
	91	mecR1Sepid_1_1	826
	92	pbp2b_1_1	805

n	Probe Name	SeqID
$\vdash$	pbp2primeSepid_1_1	806
	cat 1 1	862
	catEfaecium_1_1	861
	cmlA5_1_1	860
	ble 1 1	875
-	ddl 1 1	874
-	vanRB2 1 1	870
	vanSB2 1 1	872
	vanWB2 1 1	873
	vanXB2 1 1	876
	vanA 1 1	867
-	vanB_1_1	879
	vanC-2 1 1	881
_	vanH(tn)_1_1	866
	vanHB2 1 1	868
$\overline{}$	vanR 1 1	869
109	vanS(tn) 1 1	871
110	vanX(tn)_1_1	882
	vanY(tn)_1_1	877
		878
113	vanZ(tn)_1_1	880
114	ermA_1_1	852
115	ermB_1_2	851
116	ermC_1_1	846
117	linB_1_1	847
118	mdrSA_1_1	849
119	mefA_1_1	856
120	mphBM_1_1	855
121	mrx_1_1	857
122	msrA_1_1	854
123	satA_1_1	853
124	satSA_1_1	848
	abcXStrpmut_1_1	894
$\overline{}$	acrA_1_1	892
127	acrB_1_1	883
	acrR_1_1	890
_	albA_1_1	898
130	arr2_1_1	906
131	cadBStalugd_1_1	888
-		896
-	emeA_1_1	891
	mexA_1_1	889
	mexB_1_2	884
	mexR_1_1	905
137	mreA_1_1	907
138	norA23_1_1	904

n	Probe Name	SeqID
$\vdash$	nov_1_1	901
	gacEdelta1_1_1	895
141		893
	sul 1 1	887
	sull 1 1	886
	sulli 1 1	2888
145	wbbl_1_1	903
146	wzm_1_1	899
147	wzt_1_1	902
148	msrCb_1_1	900
149	uvrA_1_1	909
150	tetA-ACIBA_1_1	2907
151	tetAJ_1_1	863
152	tetL_1_1	864
153	tetM_1_1	865
	tetR-ACIBA_1_1	2908
155	dfrA_1_1	859
156	dfrStrpneu_1_1	858
	AAF1_1_1	247
158	ALS1_1_1	249
159	ALS7_1_1	250
160	ASL43f_1_1	232
161	BGL2_1_1	233
	CACHS3_1_1	234
163	CEF3_1_1	237
	CHS1_1_1	238
	CHS2_1_1	239
	CHS4_1_1	240
	CHS5_1_1	241
	CHT1_1_1	242
	CHT2_1_1	243
	CHT4_1_1	244
	CSA1_1_1	245
	GSC1_1_1	257
	GSL1_1_1	258
	HWP1_2_1	261
	HYR1_1_1	262
	INT1a_1_1	263
	KRE15f_1_1	264
	KRE6_1_1	265
	KRE9_1_1	266
	MP65_1_1	269
	PHR1_1_1	272
	PHR2_1_1	273
	PHR3_1_1	274
184	PRA1_1_1	275

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n	Probe Name	SeqID
185	RBT1_1_1	277
186	RBT4_1_1	278
	RHO1_1_1	279
	RVS167_1_1	283
	SKN1_1_1	285
	TCA1_1_1	287
	YAE1_1_1	289
	CDR1_1_1	911
$\overline{}$	CDR1_2_1	912
	CRD2_1_1	910
	ERG11_1_1	917
	FET3_1_1	914
	FTR2_1_1	915
	MDR1-7_1_1	916
	MET3_1_1	913
	SEC20_1_1	918
	ADH1_1_1 ARG56 1 1	248
		231
	ESS1_1_1 GAP1 1 1	253
	GAP1_1_1 GNA1_1_1	255 256
	HIS1_1_1	259
$\overline{}$		268
	NDE1_1_1	270
	PFK2_1_1	271
	SRB1_1_1	286
211	TRP1 1 1	288
-	YRB1_1_1	290
	5triphosphatase_1_1	246
=	CCT8_1_1	235
215	CDC37_1_1	236
216	EDT1_1_1	251
	ELF_1_1	252
218	FAL1_1_1	254
219	HTS1_1_1	260
$\overline{}$	MIG1_1_1	267
221	PRS1_1_1	276
222	RNR1_1_1	280
	RPB7_1_1	281
	RPL13_1_1	282
225	SHA3_1_1	284
226	YST1exon2_1_1	291
$\overline{}$	CCN1_1_1	292
	CDC28_1_1	293
	CLN2_1_1	294
230	CPH1_1_1	295

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		T
<u></u>	Probe Name	SeqID
	fliCb_1_1	144
278	nfrB_1_1	145
279	nlpA_1_1	146
280	pilAe_1_1	147
281	yacH_1_1	148
	yagX_1_1	149
	ycdS_1_1	150
_	yciQ_1_1	151
	ymcA_1_1	152
	b1202_1_1	153
	eae_1_1	154
	eltB_1_1	155
$\overline{}$	escR_1_1	156
_	escT_1_1	157
-	escU_1_1	158
_	espB_1_1	159
	—	
		160
	fes_2_1	161
	fteA_1_1	162
	hlyA_1_1	163
	hlyB_1_1	164
	iucA_1_1	165
	iucB_1_1	166
	iucC_1_1	167
	papG_1_1	168
	rfbE_1_1	169
	shuA_1_1	170
	SLTII_1_1	171
	toxA-LTPA_1_1	172
	VT2vaB_1_1	173
	ABC-eltA_1_1	317
308	agrBfs_1_1	318
309	agrCfs_1_1	319
310	arcA_1_1	308
311	arcC_1_1	309
312	bkdA_1_1	310
313	cad_1_1	311
314	camE1_1_1	312
315	csrA_1_1	313
-	dacA_1_1	314
_	dfr_1_1	315
	dhoD1a_1_1	316
	dnaE_1_1	320
_	ebsA 1 1	321
	ebsB_1_1	322
	eep_1_1	323
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n	Probe Name	SeqID
323	efaR_1_1	324
324	gls24_glsB_1_1	325
325	gph_1_1	326
	gyrAEf_1_1	327
	metEf_1_1	328
328	mntHCb2_1_1	329
$\overline{}$	mob2_1_1	330
330	mvaD_1_1	331
	mvaE_1_1	332
332	parC_1_1	333
333	pcfG_1_1	334
334	phoZ_1_1	335
	polC_1_1	336
336	ptb_1_1	337
337	recS1_1_1	338
	rpoN_1_1	339
339	tms_1_1	340
340	tyrDC_1_1	341
341	tyrS_1_1	342
342	ace_1_1	351
343	asa1_1_1	343
344	asp1_1_1	344
345	cgh_1_1	345
346	cylA_1_1	346
347	cylB_1_1	347
348	cyll_1_1	348
349	cylL_cylS_1_1	349
350	cylM_1_1	350
	ef00108_1_1	352
352	ef00109_1_1	353
353	ef0011_1_1	354
	ef00113_1_1	355
355	ef0012_1_1	356
-	ef0022_1_1	357
-	ef0031_1_1	358
	ef0032_1_1	359
	ef0040_1_1	360
360	ef0058_1_1	361
361	enlA_1_1	362
_	esa_1_1	363
$\overline{}$	esp_1_1	364
	gelE_1_1	365
	groEL_1_1	366
_	groES_1_1	367
367	<del></del>	368
368	sala_1_1	369

n Probe Name	SeqID
369 salb 1 1	370
370 sea1 1 1	371
371 sep1_1_1	372
372 vicK_1_1	373
373 yycH_1_1	374
374 yycl_1_1	375
375 yycJ_1_1	376
376 bglB_1_1	377
377 bglR_1_1	378
378 bglS_1_1	379
379 efmA_1_1	380
380 efmB 1 1	381
381 efmC_1_1	382
382 mreC_1_1	383
383 mreD 1 1	384
384 mvaDEfaecium_1_1	385
385 mvaEEfaecium_1_1	386
386 mvaK1Efaecium 1 1	387
387 mvaK2Efaecium_1_1	388
388 mvaSEfaecium 1 1	389
389 orf3 4Efaeciumb 1 1	390
390 orf6 7Efaecium 1 1	391
391 orf7 8Efaecium 1 1	392
392 orf9_10Efaecium_1_1	393
393 entA entl 1 1	394
394 entD 1 1	395
395 entR 1 1	396
396 oep_1_1	397
397 sagA_1_2	398
398 H+ATPase_1_1	2887
399 cymA_1_1	449
400 cymD_1_1	450
401 cymE_1_1	451
402 cymH_1_1	452
403 cyml_1_1	453
404 cymJ_1_1	454
405 ddrA 1 1	455
406 fdt-1 1 1	456
407 fdt-2 1 1	457
408 fdt-3 1 1	458
409 gatY_1_1	459
410 hydH_1_1	460
411 masA_1_1	461
412 nasA_1 1	462
413 nasE_1_1	463
414 nasF_1_1	464

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n	Probe Name	SeqID
415	pehX_1_1	465
416	pelX_1_1	466
417	tagH_1_1	467
$\overline{}$	tagK_1_1	468
419	tagT_1_1	469
420	acoA_1_1	408
421	acoB_1_1	409
422	acoC_1_1	410
423	ahlK_1_1	411
424	atsA_1_1	399
425	atsB_1_1	400
426	budC_1_1	401
427	citA_1_1	402
428	citW_1_1	403
429	citX_1_1	404
430	dalD_1_1	405
431	dalK_1_1	406
432	dalT_1_1	407
433	fimK_1_1	412
434	glfKPN2_1_1	413
435	liac_1_1	431
436	ltrA_1_1	414
437	mdcC_1_1	415
438	mdcF_1_1	416
439	mdcH_1_1	417
440	mrkA_1_1	418
441	mtrK_1_1	419
442	nifF_1_1	420
$\overline{}$	nifK_1_1	421
	nifN_1_1	422
445	tyrP_1_1	423
_	ureA_1_1	424
_	wbbO_1_1	425
	wza_1_1	426
	wzb_1_1	427
	wzmKPN2_1_1	428
	wztKPN2_1_1	429
	yojH_1_1	430
	aldA_1_1	433
$\overline{}$	aldA_2_1	434
455		432
$\overline{}$	hemly_1_1	435
_	pSL017_1_1	436
	pSL020_1_1	437
	rcsA_1_1	438
460	rmlC_1_1	439

n	Probe Name	SeqID
461	rmiD_1_1	440
_	waaG_1_1	441
_	wbbD 1 1	442
	wbbM_1_1	443
-	wbbN_1_1	444
	wbdA_1_1	445
	wbdC 1 1	446
$\overline{}$	wztKpn_1_1	447
	yibD_1_1	448
	glpR_1_1	470
	lasRb 1 1	471
	OrfX_1_1	472
	pa0260_1_1	473
_	pa0572 1 1	474
	pa0625_1_1	475
	pa0636 1 1	476
	pa1046_1_1	477
	pa1069 1 1	478
	pa1846_1_1	479
_	pa3866 1 1	480
	pa4082_1_1	481
	pilAp_1_1	482
	PilAp2_1_1	483
484	pilC_1_1	484
_	PstP 1 1	485
_	purK_1_1	486
_	uvrDII 1 1	487
	vsml 1 1	488
	vsmR_1_2	489
	xcpX_1_1	490
	algB_1_1	494
	 algN_1_1	495
	algR_1_1	496
	aprA_1_1	491
	aprE_1_1	492
496		493
	ExoS_1_1	497
$\vdash$	fpvA_1_1	498
	lasRa_1_1	499
500		500
	lipH_1_1	501
	Orf159_1_2	502
_	Orf252 1 1	503
	pchG_1_1	504
	PhzA 1 1	505
	PhzB 1 1	506
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n	Probe Name	SeqID
_	PLC_1_1	507
_	plcN_1_1	508
	plcR_1_1	509
	pvdD_1_1	510
	pvdF_1_2	511
	pyocinS1_1_1	512
	pyocinS1im_1_1	513
	pyocinS2_1_1	514
_	pys2_1_1	515
	pys2_2_1	516
	rbf303 1 1	517
518	rhiA 1 1	518
519	rhlB_1_1	519
520	rhIR_1_1	520
	TnAP41_1_2	521
522	toxA_1_1	522
523	aad_1_1	711
524	atfA_1_1	706
525	atfB_1_1	707
526	atfC_1_1	708
	ccmPrmi1_1_1	709
-	cyaPrmi_1_1	710
	flfB_1_1	712
	flfD_1_1	713
	flfN_1_1	714
	flhD_1_1	715
	floA_1_1	716
	ftsK_1_1	717
	gstB_1_1	718
	hemCPrmi_1_1	719
	hemDPrmi_1_1	720
538	hev_1_1 katA 1 1	721
		722
	lpp1_1_1 menE 1 1	723 724
	mfd_1_1	<del></del>
	nrpA_1_1	725 726
_	nrpB_1_1	727
	nrpG_1_1	728
	nrpS_1_1	729
	nrpT_1_1	730
	nrpU_1_1	731
_	pat_1_1	732
	pmfA_1_1	733
	pmfC_1_1	734
	pmfE_1_1	735
تت	·	

n	Probe Name	SeqID
553	ppaA_1_1	736
554	rsbA_1_1	737
555	rsbC_1_1	738
556	speB_1_1	739
557		740
	stmB_1_1	741
	terA_1_1	742
-	terD_1_1	743
561	umoA_1_1	744
	umoB_1_1	745
	umoC_1_1	746
_	ureR_1_1	747
-	xerC_1_1 vabA 1 1	748
	73	749
	flaA_1_1 flaD 1 1	750
569		751 752
	hpmA_1_1	752 753
	hpmB_1_1	753 754
$\overline{}$	lpsPrmi 1 1	755
_	mrpA_1_1	756
-	mrpB_1_1	757
	mrpC_1_1	758
	mrpD_1_1	759
$\overline{}$	mrpE_1_1	760
	mrpF_1_1	761
579	mrpG_1_1	762
580	mrpH_1_1	763
581	mrpl_1_1	764
582	mrpJ_1_1	765
583	patA_1_1	766
584	putA_1_1	767
$\overline{}$	uca_1_1	768
	ureDPrmi_1_1	769
	ureEPrmi_1_1	770
$\vdash$	ureFPrmi_1_1	771
	zapA_1_1	772
	zapB_1_1	773
	zapD_1_1	774
	zapE_1_1	775
	envZPrvu_1_1	776
$\vdash$	frdC_1_1 frdD 1 1	777
		778
		779
	lad_1_1 tna2 1 1	780 781
290	uiaz_1_1	781

n	Probe Name	SeqID
599	end_1_1	782
600	pqrA_1_1	783
601	urg_1_1	784
602	eD_2_1	2892
603	eE_1_1	2890
604	eF_1_1	2899
605	et_1_1	2898
606	ORF2-STEMA_1_1	2897
607	ORF4-STEMA_1_1	2896
608	pam_1_1	2895
609	pmp-STEMA_1_1	2894
610	ppi_1_1	2893
611	smeE_1_1	2889
612	smeF4494_1_1	2901
613	StmPr1_1_1	2891
614	StmPr2_1_1	2900
615	0487Straga_1_1	625
616	0488Straga_1_1	626
617	0493Straga_1_1	627
618	0495Straga_1_1	628
619	0498Straga_1_1	629
620	0500Straga_1_1	630
621	0502Straga_1_1	631
622	0504Straga_1_1	632
623	cpsA1Strgal_1_1	606
624	cpsB1Strgal_1_1	607
625	cpsC1Strgal_1_1	608
626	cpsD1Strgal_1_1	609
627	cpsE1Strgal_1_1	610
628	cpsG1Strgal_1_1	611
629	cpsIStragal_1_1	612
630	cpsJStragal_1_1	613
631	cpsKStragal_1_1	614
632	cpsMStragal_1_1	615
633	cpsYStragal_1_1	616
634	cpsYStragal_2_1	617
635	cylBStraga_1_1	618
636	cylEStraga_1_1	619
637	cylFStraga_1_1	620
638	cylHStraga_1_1	621
639	cyllStraga_1_1	622
	cylJStraga_1_1	623
641	cylKStraga_1_1	624
-	folDStraga_1_1	633
643	neuA1Strgal_1_1	634
644	neuB1Strgal_1_1	635

690 immunofrag3Strpneu\_2\_1

n Probe Name	SeqID
691 kdtBStrpneu_1_1	560
692 lysAStrpneu_1_1	561
693 pcpBStrpneu_1_1	562
694 pflCStrpneu 1 1	563
695 plpA_1_1	564
696 prtA1Strpneu_1_1	565
697 pspC1Strpneu_1_1	566
698 pspC2_1_1	567
699 purRStrpneu 1 1	568
700 pyrDAStrpneum_1_1	569
701 SP0828Strpneu_1_1	570
701 SP00203tipfied_1_1	571
703 SP0833Strpneu_1_1	572
704 SP0837 38Strpneu_1_1	573
705 SP0839Strpneu_1_1	574
706 ugdStrpneu_1_1	575
707 uncC 1 1	576
708 vicXStrepneu_1_1	577
709 wchA6bStrpneum_1_1	578
710 wci4Strpneum_1_1	579
711 wciK4Strpneum_1_1	580
712 wciL4Strpneum_1_1	581
713 wciN6bStrpneum_1_1	582
714 wciO6bStrpneum_1_1	583
715 wciP6bStrpneum_1_1	584
716 wciY18Strpneum_1_1	585
717 wzdbStrpneum_1_1	586
718 wze6bStrpneum_1_1	587
719 wzy18Strpneum_1_1	588
720 wzy4Strpneum_1_1	589
721 wzy6bStrpneum 1_1	590
722 xpt_1_1	591
723 igaStrpneu_1_1	592
724 lytA_1_1	593
725 nanA_1_1	594
726 nanBStrpneu_1_1	595
727 pcpCStrpneu_1_1	596
728 ply_1_1	597
729 prtAStrpneu_1_1	598
730 pspA_1_2	599
731 SP0834Strpneu_1_1	600
732 SP0834Strpneu_1_2	601
733 sphtraStrpneu_1_1	602
734 wciJStrpneu_1_1	603
735 wziyStrpneu_1_1	604
736 wzxStrpneu_1_1	605

	_
n Probe Name	SeqID
737 cyclStrpyog_1_1	645
738 fah_rph_hlo_Strpyog_1_1	646
739 int_1_1	647
740 int315.5 1 1	648
741 murEStrpyog_1_1	649
742 oppA_1_1	650
743 oppCStrpyog_1_1	651
744 oppD_1_1	652
745 SPy0382Strpyog_1_1	653
746 SPy0390Strpyog_1_1	654
747 SpyM3_1351_1_1	655
748 vicXStrpyog_1_1	656
749 DNaselStrpyog_1_1	657
750 fba2Strpyog_1_1 751 fhuAStrpyog 1 1	658
	659
752 fhuB1Strpyog_1_1	660
753 fhuDStrpyog_1_1	661
754 fhuGStrpyog_1_1	662
755 hylA_1_1	663
756 hylP_1_1	664
757 hylp2_1_1	665
758 oppB_1_1	666
759 ropB_1_1	667
760 scpAStrpyog_1_1	668
761 sloStrpyog_1_1	669
762 smez-4Strpyog_1_1	670
763 sof_1_1	671
764 sof_2_1	672
765 speA_1_1	673
766 speB2Strpyog_1_1	674
767 speCStrpyog_1_1	675
768 speJStrpyog_1_1	676
769 srtBStrpyog_1_1	677
770 srtCStrpyog_1_1	678
771 srtEStrpyog_1_1	679
772 srtFStrpyog_1_1	680
773 srtGStrpyog_1_1	681
774 srtlStrpyog_1_1	682
775 srtKStrpyog_1_1	683
776 srtRStrpyog_1_1	684
777 srtTStrpyog_1_1	685
778 vicKStrpyog_1_1	686
779 573Stprmut_1_1	687
780 580SStprmut_1_1	688
781 581_582SStprmut_1_1	689
782 584SStprmut_1_1	690

n	Probe Name	SeqID
$\vdash$	dltAStrmut_1_1	691
784	dltBStrmut_1_1	692
785	dltCppx1Strmut_1_1	693
	dltDStrmut_1_1	694
	lichStrbov_1_1	695
	lytRStprmut_1_1	696
$\vdash$	lytSStprmut_1_1	697
	pepQStrrmut_1_1	698
	pflCStrmut_1_1	699
	recNStprmut_1_1	700
_	ytqBStrmut_1_1	701
	hlyXStrmut_1_1	702
	igaStrmitis_1_1 igaStrsanguis 1 1	703 704
	<u> </u>	
	perMStrmut_1_1 fasCAXStrdysg_1_1	705 2904
	sloStrep_1_1	2905
	cataSaur_1_1	2903
$\overline{}$	cataSaur_1_1	2
_	clfA_1_1	3
	clfB_1_1	3 4
_	coa_1_1	5
	coa_1_2	6
$\overline{}$	coa_2_2	2903
807	coa_3_1	2902
808	epiP-bsaP_1_1	58
809	geh_1_1	59
	gyrA_1_1	60
	gyrB_1_1	61
-	hemB_1_1	62
	hemC_1_1	63
	hemD_1_1	64
$\overline{}$	hemN_1_1	65
<del>-</del>	hsdS_1_1	66
<b>——</b>	hsdS_2_1	67
_	lip_1_1	68
$\overline{}$	menC_1_1	69 70
	murC_1_1 nuc_1_1	70 71
<del></del>	pdhD_1_1	71
-	rpoB_1_1	73
_	SAV0431 1 1	74
	SAV0431_1_1 SAV0439_1_1	75
	SAV0440_1_1	76
-	SAV0441 1 1	77
	sigB_1_1	
828	sigB_1_1	78

n	Probe Name	SeqID
829	spa_1_2	79
	sstC_1_1	80
831		81
	tyrA_1_1	82
-	bsaE 1 1	100
	bsaG_1_1	101
	cap5h_1_1	102
-	cap5i_1_1	102
	cap5i_1_1	103
	<del></del>	105
)	cap8H_1_1	106
	cap8I_1_1	107
	cap8J_1_1	108
_	cap8K_1_1	109
$\blacksquare$	EDIN_1_1	113
844		114
845	etb_1_1	115
846	hglA_1_1	116
847	hglA_2_1	117
848	hglB_1_1	118
849	hglC_2_1	119
850	hla_1_1	120
851		121
852	lukF_1_1	122
	lukS 1 1	123
	lukS_2_1	124
	NAG 1 1	125
856		126
857		127
858		128
$\overline{}$	sec1_1_1	129
		130
000		Ť
$\vdash$	seh_1_1	131
_	sel_1_1	132
	set15_1_1	133
	set6_1_1	134
_	set7_1_1	135
	set8_1_1	136
_	sprV8_1_1	137
868		138
	agrB_1_1	178
	agrC_1_1	179
871	alphSE1368_1_1	180
872	ardeSE0106_1_1	174
873	ardeSE0107_1_1	175
874	aroiSE0105_1_1	176

n	Probe Name	SeqID
$\vdash$	atlE_1_1	177
$\vdash$	gad_1_1	181
	glucSE1191_1_1	182
$\overline{}$	hsp10 1 1	183
	icaA_1_1	184
	icaB 1 1	185
	mvaSSepid_1_1	186
882	nitreSE1972_1_1	187
	nitreSE1974 1 1	188
	nitreSE1975 1 1	189
885	oiamtSE1209_1_1	190
	ORF1Sepid_1_1	191
	ORF3bSepid_1_1	192
	qacR_1_1	193
889		194
-	ureSE1861_1_1	195
	ureSE1863_1_1	196
	ureSE1864_1_1	197
893	ureSE1865_1_1	198
894	ureSE1867 1 1	199
895	gcaD_1_1	200
	hld_orf5_1_1	201
897	icaC_1_1	202
898	icaD_1_1	203
899	icaR_1_1	204
900	psm_beta1and2_1_1	205
901	purR_1_1	206
902	spoVG_1_1	207
	yabJ_1_1	208
904	folQShaemolyt_1_1	209
905	mvaCShaemolyticus_1_1	210
906	mvaDShaemolyt_1_1	211
	mvaK1Shaemolyticus_1_1	212
908	mvaSShaemolyticus_1_1	213
909	RNApolsigm_1_1	214
910	lipShaemolyt_1_1	215
911	ydhK_1_1	2906
912	agrB2Stalugd_1_1	216
913	agrC2Stalugd_1_1	217
914	agrCStalugd_1_1	218
915	slamStalugd_1_1	219
916	fblStalugd_1_1	220
917	slushABCStalugd_1_1	221
918	RNApolsigmSsapro_1_1	222
919	RNApolsigmSsapro_1_2	223
920	msrw1Stwar_1_1	224

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n	Probe Name	SeqID
921	nukMStwar_1_1	225
922	proDStwar_1_1	226
923	proMStwar_1_1	227
924	sigrpoStwar_1_1	228
925	tnpStwar_1_1	229
	gehAStwar_1_1	230
927	0135mihck_1_1	945
	0270cap_1_1	947
929	FAN_1_1	946
930	p53_1_1	944

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All genes were selected from the literature and databases, compared by BLAST analysis to all other sequences available in the NCBI database. Primers were designed to amplify gene segments of 200 to 800 bp length devoid of apparent homology with genes of other bacterial species and *Homo sapiens*. Gene segments were amplified by using the puReTaq Ready-To-Go PCR beads (Amersham Biosciences, Freiburg, Germany) and cloned into the pDrive Cloning Vector (Qiagen, Hilden, Germany) according to the recommendations of the suppliers and transformed into competent *Escherichia coli* (XL-1-Blue) cells using the calcium chloride protocol (Sambrook, J. and Russell, D.W. 2001. Molecular cloning: a laboratory manual, 3<sup>rd</sup> ed. Cold Spring Harbor Laboratory Press, New York, N.Y).

For quality control purposes, all gene probes were partially sequenced and verified (with the BigDye kit 1.1 and an 377 DNA sequencer; Applied Biosystems, Foster City, USA). All sequences obtained were identical or substantially identical (>90% sequence identity) to those obtained from the database.

For DNA-probe production 930 recombinant plasmids containing the 930 selected gene segments were used for re-amplification. Amplicons were purified and spotted in 4 replicates per slide on UltraGAPS™ Coated Slides (gamma amino propyl silane coated slides, Corning, NY, USA). Approximately 1 nl DNA (with a concentration of about 0.1 to about 0.2 ng/nl) per spot was spotted onto the slide with a Biorobitics Microgrid Microarrayer (Genomic Solutions, Ann Arbor, MI, USA).

#### Example 2.5: Hybridization and scanning

All experiments described represent dual co-hybridizations of two different target DNA samples labelled respectively with Cy3 or Cy5. After removal of unbound label, Cy3 and Cy5 labelled DNAs were pooled and mixed with 10 µg of Salmon Sperm

DNA and 50 µg of poly-A-DNA. The mixture was frozen in liquid nitrogen and lyophilized in the dark. Prior to hybridization the target DNA was reconstituted in 110 µl hybridization solution (30% formamide, 0.1% SDS, 5xSSC) and denatured by heating at 95°C for 3 min prior to hybridization. Hybridization was automatically performed with a TECAN Hybridization Station (HS400, TECAN, Salzburg, Austria). The arrays were prewashed at 42°C for 1 min with 5x SSC and prehybridized in 110 µl denatured prehybridization buffer (30% formamide, 0.1% SDS, 5xSSC, 10mg/ml BSA) for 30 min at 42°C at mild agitation. After injection of 110 µl labelled DNA, hybridization was performed at 60°C for 18 hours at medium agitation. The arrays were washed at 42°C in wash buffer I (1x SSC, 0.1% SDS) - three cycles of 30 sec wash time and 2 min soak time -, in wash buffer II (0.1x SSC, 0.1% SDS) - five cycles of 30 sec wash time and 2 min soak time - and wash buffer III (0.1x SSC) four cycles of 30 sec wash time and 2 min soak time - and finally dried at 30°C with N<sub>2</sub> (2.7 bar) for 3 min. Hybridized arrays were scanned with GenPix Personal Axon 4100A laser scanner (Axon Instruments, Union City, CA, USA). Laser light of wavelengths at 532 and 635 nm was used to excite Cy3 dye and Cy5 dye, respectively. Fluorescent images were analyzed by the GenePix Pro 6.0 and Acuity 4.0 software (Axon Instruments). For each feature (gene probe) the median pixel intensity of wavelength 635 nm or 532 nm, respectively, was determined and the median background of the respective wavelength subtracted (F635 Median - B635 and F532 Median - B532, respectively).

#### Example 2.6: Specificity

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In order to allow the simultaneous and rapid identification, differentiation and characterisation of pathogens causing sepsis, a microarray comprising a set of 930 gene probes of 200 to 800 bp length was developed (Tab. 7). The clinically most relevant sepsis causing pathogens were represented on the microarray by gene probes specific for the genera and species *E. coli* (31), *Staphylococcus aureus* (69) and coagulase negative staphylococci (58), *P. aeruginosa* (53), *Streptococcus* spp. (185), *Enterococcus* spp.(92), *Proteus* spp. (79), *Klebsiella* spp.(71), *Enterobacter* spp. (11), *Stenotrophomonas maltophilia* (13), *Acinetobacter baumannii* (21) and *Candida albicans* (86). To allow for parallel detection of antibiotic resistance determinants, the array contained 131 bacterial resistance gene probes.

To facilitate the optimization, validation and standardization of microarray analysis, a set of 29 control probes was included. Different 16S rRNA gene probes (18) served as positive hybridization controls for bacterial DNA. The gene probe rbcL\_1\_2 (segment of the rubisco gene of *Hordeum vulgaris*) was prelabelled with Cy3 and Cy5 and spotted onto each subarray for visualisation of the array orientation. Gene probes derived from *Mus musculus* (2), *Dictyostelium discoideum* (2), *Homo sapiens* (5), *Hordeum vulgaris* (1) were included as negative or positive hybridization controls. In all assays, one to five PCR-amplified DNA-segments, which had been added to each DNA preparation as a positive control, hybridized with the corresponding probes, indicating that labelling and hybridization had performed efficiently.

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The specificity of the DNA-chip was validated with 44 well characterized clinical isolates and reference strains of the target species (40) as well as other related bacteria (4) (Table 8).

<u>Tab. 8:</u> Microorganism strains used for microarray validation. Non-target species are Nos 21, 25, 27 and 30.

No	Species	Strain	Dye
1	A. baumannii	DSM 30008	Cy5
2	A. baumannii	5256-2	СуЗ
3	P. aeruginosa	ATCC 27853	Су3
4	E. coli	CIP 105893	СуЗ
5	E. coli	ATCC 25922	Cy5
6	E. coli	CIP 81.88	СуЗ
7	E. coli	CIP 74.14	Cy5
8	E. coli	U10338-1	Cy5
9	E. coli	U10164-2	Cy5
10	E. coli	U10248-1	Cy5
11	K. oxytoca	DSM 4798	Cy5
12	K. oxytoca	U10274	Cy5
13	K. pneumoniae	DSM 681	СуЗ
14	K. pneumoniae	O3-390	СуЗ
15	K. pneumoniae	O8-889	СуЗ
16	P. mirabilis	DSM 788	Cy5
17	P. mirabilis	U10515	Cy5
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18	P. mirabilis	U9979-1	Cy5
19	P. vulgaris	DSM 2140	Cy5
20	C. albicans	ATCC 10231	СуЗ
21	E. casseliflavus	UW703/95	Cy5
22	E. faecalis	ATCC 29212	Cy5
23	E. faecalis	UW700/95	Cy5
24	E. faecium	VRE 9182	СуЗ
25	E. gallinarum	UW701/97	СуЗ
26	S. agalactiae	DSM 2134	Cy5
27	S. angiosus	DSM 20563	СуЗ
28	S. bovis	DSM 20480	Cy3
29	S. dysgalactiae	DSM 20662	Cy3
30	S. gordonii	DSM 6777	Cy5
31	S. mutans	DSM 20523	Су3
32	S. pneumoniae	ATCC 49619	СуЗ
33	S. pyogenes	DSM 11723	Cy3
34	S. aureus	ATCC 29213	СуЗ
35	S. aureus	P2716	Cy3
36	S. aureus	C5010	Cy3
37	S. aureus	MW2	Cy3
38	S. epidermidis	ATCC 12228	Cy5
39	S. epidermidis	BC 1920	Cy5
40	S. haemolyticus	DSM 20263	Cy5
41	S. hominis	DSM 20228	Cy5
42	S. lugdunensis	DSM 4804	CY3
43	S. saprophyticus	ATCC 14953	СуЗ
44	S. warneri	DSM 20316	Cy5

Hybridization experiments with DNA obtained from the respective target strains revealed hybridization profiles specific for the different species and genera (Fig. 7). In contrast, non-target organisms hybridized nearly exclusively with 16S rRNA (Probe Nos. 1-24) and antibiotic gene probes (Probe Nos. 26-156) (Fig. 7 panels G and H).

Example 2.7: Specificity of hybridization profiles for fungi

DNA of the fungus *Candida albicans* hybridized specifically with the *Candida* gene probes (Probe Nos. 157-242) including *Candida* resistance probes but not with bacterial 16 rRNA or species specific probes (Fig. 8, panel A). The specificity of two selected *Candida* probes is demonstrated in Fig. 8 panel B, the probes *ALS1* and *ASL43f* hybridized only with DNA obtained from *C. albicans* and not with any DNA obtained from the 43 bacterial strains.

#### Example 2.8: Specificity of hybridization profiles for Gram-negative bacteria

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Strains of the genus Klebsiella showed specific hybridization with the Klebsiella gene probes (Probe Nos. 399-469). For this genus cross hybridization with lower intensity of the fluorescent signals was observed with some E. coli and P. aeruginosa probes (Nos. 275-306 and 470-522, respectively). This is also the case for bacterial strains of the genus Proteus, which show major hybridization with the Proteus gene probes allowing unambiguous identification (Probe Nos. 523-601). Vice versa, P. aeruginosa and E. coli can be easily identified by their hybridization profiles, but show minor cross hybridization with gene probes of Klebsiella, E. coli and P. aeruginosa, respectively. The E. coli reference strain CIP 105893 and the clinical isolate U10164-2 show nearly identical hybridization profiles, demonstrating the high reproducibility of the assay. Strains of the non-fermenting Gram-negative bacterium A. baumannii were readily identified based on their microarray hybridization profile showing specific hybridization to the A. baumannii gene probes (Nos. 243-263). The specificity of selected species specific probes is shown in Figure 9. The A. baumannnii probe csuA hybridized only with labelled DNA preparations derived from A. baumannnii strain DSM 30008 and the clinical A. baumannii isolate but not with any other of the 42 strains. The P. aeruginosa probe PhzA showed hybridization signals with a high intensity >60000 (Median fluorescence - background) only with DNA of the P. aeruginosa reference strain but with no other pathogen, demonstrating that although some P. aeruginosa probes show cross-hybridization with other Gram-negative species, unambiguous identification is feasible. Equally specific results were obtained with the E. coli probe shuA, which showed significant hybridization signals > 40000 only with DNA of the seven E. coli reference strains and clinical isolates. The closely related species K. oxytoca and K. pneumoniae were easily identified and discriminated from each other by the K. oxytoca probe tagK and the K. pneumoniae

probe *acoC*. The *P. mirabilis* probe *hpmB* was highly specific for the three *P. mirabilis* strains and isolates, while probe *enzZPrvu* was specific for *P. vulgaris*.

## Example 2.9: Specificity of hybridization profiles for Gram-positive bacteria of the genus *Enterococcus*

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The microarray assay was highly specific in the identification of Gram-positive target species. Clinical isolates of the species *E. faecalis* and *E. faecium* could be identified and discriminated unambiguously by their hybridization profiles (Probe Nos. 307-375 and 376-398, respectively) (Fig. 7, panels E and F). The vancomycin resistant non-target strain *E. casseliflavus* (Fig. 7, panel G) showed hybridization to the bacterial 16S rRNA probes, the antibiotic resistance gene probes *vanC-2* (vancomycin resistance), *arr2* (Rifampin resistance) and *tetM* (tetracycline resistance) and the *S. aureus* probes *gyrA* (DNA gyrase subunit A), *rpoB* (RNA polymerase B subunit) and *sstC* (iron transport protein) only. This profile does not permit species identification but indicates a vancomycin resistant bacterium. A similar profile was obtained for the vancomycin resistant non-target strain *E. gallinarum* (not shown).

### Example 2.10: Specificity of hybridization profiles for Gram-positive bacteria of the genus *Streptococcus*

Microarray hybridization assays performed with streptococcal DNA obtained from reference strains of *S. pneumoniae*, *S. pyogenes*, *S. mutans* and *S. agalactae* revealed species specific hybridization profiles and an excellent identification and discrimination of these target organisms (Fig. 7). The species *S. dysgalactiae* and *S. bovis* (*S. viridans* group) are each represented by a single gene probe on the array (fasCAXStrdysg and lichStrbov, respectively). These probes however exhibited specific hybridization to the target DNA only, and in this way permitted identification of the two species. Additionally both species showed hybridization with the 16S rRNA gene probes and *pbp2b* (penicillin binding protein of *S. pneumoniae*). Furthermore, *S. dysgalactiae* DNA hybridized with the probes *dacCStrpyog* and *murEStrpyog* and *S. bovis* DNA with *gyrA*, *rpoB* and *sstC* as *E. casseliflavus*. The non-target species *S. gordonii* and *S. angiosus* were readily discriminated by their hybridization profiles from other streptococci, *S. gordonii* 

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showed hybridization to the 16S rRNA genes only, *S. angiosus* DNA hybridized additionally to *gyrB* and *rpoB* (Fig. 7 H).

## Example 2.11: Specificity of hybridization profiles for Gram-positive bacteria of the genus *Staphylococcus*

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Hybridization assays performed with S. aureus strains and S. epidermidis DNA produced very specific hybridization profiles with little cross hybridization (Fig. 7 AB). The specificity of selected probes for coagulase-negative staphylococci is shown in Fig. 10. S. saprophyticus, S. haemolyticus, S. lugdnunensis, S. warneri and S. hominis produced hybridization profiles distinct of those from S. aureus and S. epidermidis. For these species the following species specific probes were detected: RNAposigmSsapro\_1 and \_2 for S. saprophyticus, RNApolisigm and mvaDShaemolyt for S. haemolyticus, agrCStalugd, slamStalugd and fblStalug for S. lugdunensis and proDStwar, gehAStwar and msrw1Stwar for S. warneri. For S. hominis no probe proved to be species specific. The S. hominis derived probe ydhK cross hybridized with DNA of S. hominis, S. epidermidis and S. haemolyticus. However, certain probe patterns seem to be species specific for S. hominis and may allow identification and discrimination from S. haemolyticus and other CoNS (eg. hybridization of ydhK, tnpStwar and sin and absence of mvaDShaemolyt and RNApolsigm).

# Example 2.12: Detection of antibiotic resistance determinants in Gram-negative bacteria

Susceptibility results determined by the VITEK2 system were compared to the results of the microarray hybridization assay for the simultaneous detection of antibiotic resistance genes.

For the Gram-negative enterobacteria *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis* and *P. vulgaris* there was a 100% correlation between phenotypic resistance to aminoglycosides (Gentamycin, Tobramycin) and hybridization to at least one of the aminoglycoside gene probes *aacA4*, *aacC2*, *aadA*, *aacA* and *aphDStwar* (Table 9).

<u>Tab. 9: Aminoglycoside resistance of Gram-negative enterobacteria:</u>

Strain	Aminoglycoside	Aminoglycoside

	resistance phenotype <sup>a</sup>	resistance gene
		<u>-</u>
E. coli CIP 105893	GENI, TOB	aacA4, aadA
E. coli ATCC 25922	susceptible	-
E. coli CIP 81.88	susceptible	-
E. coli CIP 74.14	STR	_
E. coli U10338-1	GENI, TOB	aacA4
E. coli U10164-2	GEN, TOB	aacC2
E. coli U10248-1	GEN, TOB	aacC2, strB
K. oxytoca DSM 4798	susceptible	-
K. oxytoca U10274	susceptible	-
K. pneumoniae DSM 681	susceptible	-
K. pneumoniae 390	susceptible	-
K. pneumoniae 889	susceptible	strB
P. mirabilis DSM 788	susceptible	-
P. mirabilis U10515	susceptible	aacC1
P. mirabilis U9979-1	GEN, TOB	aacC2, aadA,
		aacA_aphDStwar, strB
P. vulgaris DSM 2140	susceptible	-

<sup>&</sup>lt;sup>a</sup>GEN Gentamycin; TOB tobramycin; STR Streptomycin, resistance was not tested routinely; i, intermediary resistance

All enterobacterial strains which showed resistance to β-lactam antibiotics (penicillin and cephalosporines) hybridized with at least one or more β-lactamase gene probes (blaCTX-M, blaFOX-3 and -6, blaPRMI, blaTEM, blaSHV, blaOXY-KLOX, blaA) (Table 10). There was no hybridization with the resistance gene probes ampC and blaOXA with any of the tested strains.

10 <u>Tab. 10: β-lactam resistance of Gram-negative enterobacteria:</u>

Strain	ß-lactam resistance	ß-lactamase genotype <sup>b</sup>
	phenotype <sup>a</sup>	
E. coli CIP 105893	ESBL	blaCTX-M-22, blaFOX-3,
		blaFOX-6, blaPRMI, blaTEM
E. coli ATCC 25922	susceptible	_
E. coli CIP 81.88	susceptible	_
E. coli CIP 74.14	susceptible	-
E. coli U10338-1	ESBL	blaCTX-M-22, blaTEM

E. coli U10164-2	ESBL	blaCTX-M-22, blaOXY, blaPRMI,
		blaTEM
E. coli U10248-1	AMP, ASU, MEZ, PRLi,	blaCTX-M-22, blaPRMI, blaSHV,
	TZPi, CXM	blaTEM
K. oxytoca DSM 4798	AMP, ASUi, MEZi	blaOXY
K. oxytoca U10274	ESBL	blaCTX-M-22, blaOXY, blaOXY-
		KLOX, blaSHV
K. pneumoniae DSM 681	AMP, ASUi, MEZi, PRLi	blaCTX-M-22, blaFOX-3,
		blaFOX-6, blaOXY, blaSHV
K. pneumoniae 390	AMP, ASUi, MEZi	blaCTX-M-22, blaFOX-3,
		blaFOX-6, blaOXY, blaOXY-
		KLOX, blaSHV
K. pneumoniae 889	AMPi	blaCTX-M-22, blaFOX-3,
		blaFOX-6,blaOXY-KLOX,
		blaSHV
P. mirabilis DSM 788	KZi, CXMi, IMP	~
P. mirabilis U10515	ESBL,IMP	blaCTX-M-22,
P. mirabilis U9979-1	ESBL, IMP	blaCTX-M-22, blaFOX-3,
		blaFOX-6, blaOXY, blaPRMI,
		blaTEM
P. vulgaris DSM 2140	AMP, KZ	blaA <sup>d</sup>

<sup>&</sup>lt;sup>a</sup>ESBL extended spectrum β-lactamases; AMP, Ampicillin; ASU, Ampicillin/Sublactam; MEZ, Mezlocillin; PRL, Piperacillin; KZ, Cefazolin; CXM, Cefuroxim; IMP, Imipenem; i, intermediary resistance

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Strains susceptible to  $\beta$ -lactam antibiotics did not show significant hybridization signals (Median fluorescence – background <10000) with any of the  $\beta$ -lactamase gene probes. Although the hybridization pattern permitted the detection of different types of  $\beta$ -lactamases (blaTEM, blaSHV, blaCTX-M, blaFOX), it did, however, not allow the detection and discrimination of extended spectrum  $\beta$ -lactamases (ESBL). For the two clinical isolates of P. mirabilis the ESBL phenotype was correlated with hybridization of the acrA, -B and -R genes, which encode a multidrug efflux pump. Furthermore, for these two species, resistance to tetracycline was correlated with hybridization of the P. mirabilis derived gene probe tetAJ.

<sup>&</sup>lt;sup>b</sup>Fluorescence signals ≥10000 were considered positive.

<sup>&</sup>lt;sup>c</sup>Fluorescence <10000; most fluorescence signals were <30000 for the hybridization assay with *P. vulgaris* DMS 2140

### Example 2.13: Detection of antibiotic resistance determinants in Gram-positive bacteria

The phenotypic vancomycin resistance of the tested enterococci correlated by 100% with the genotypic resistance determined by microarray hybridization (Table 11).

Tab. 11: Phenotypic and genotypic resistance of Enterococcus strains.

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Strain	Resistance	Resistance	1			
	phenotype	genotype				
		Aminoglycosides	Glycopeptides	Macrolides	Tetracycline	Efflux pumps
E. casseliflavus UW703/95	VAN, DA. QDi	-	vanC	-	tetM	-
E. faecalis ATCC 29212	DA, Ei, QD, TET, SXT	-	-	-	tetM	emeA
E. faecalis UW700/95	VAN, DA, E, GEN, QD, STR, SXT	aacA-aphD	vanB	ermB	-	emeA <sup>b</sup>
<i>E. faecium</i> VRE9182	VAN, AMPI, DA, E, QDI, STR, Teicoplanin, TET	aphA3 <sup>b</sup>	vanA, vanB	ermB	tetL, tetM	msrCb
E. gallinarum UW701/97	VAN, DA, QDi, SXT, TET	-	vanC	-	tetM	-

<sup>a</sup>VAN, vancomycin; DA, clindamycin; E, erythromycin; QD, quinupristin/dalfopristin (streptogramins); STR, streptomycin, TET, tetracycline; i, intermediary resistance. <sup>b</sup>Relative low fluorescence intensity (Median fluorescence – background <18.000).

Hybridization to the *vanC-2* gene was observed for the two vancomycin resistant strains *E. casseliflavus* and *E. gallinarum*, which contain the *vanC-2* and the *vanC-1* gene, respectively. The *vanB* gene was detected in the clinical isolates of *E. faecalis* UW700/95 and *E. faecium* VRE9182, the latter strain also hybridized with the *vanA* gene, indicating the presence of both genes. Furthermore, these two strains showed hybridization with aminoglycoside resistance genes (*aacA-aphD* and *aphA3*, respectively) and the macrolide resistance gene *ermB* (Table 11). The presence of efflux pumps involved in macrolide resistance was indicated by microarray hybridization for both *E. faecalis* strains (*emeA*) and *E. faecium* VRE9182 (*msrCb*).

Genotypic resistance to tetracycline was detected for four of the five strains (hybridization to *tetL* and/or *tetM*).

The tested streptococci showed phenotypic susceptibility to all tested antiobitics.

For staphylococci, there was 100% correlation between phenotypic resistance to penicillin and hybridization of the *blaZ* and the *blaIShaemolyt* gene probes and between oxacillin resistance and hybridization to the *mecA* gene (Table 12).

Tab. 12: Phenotypic and genotypic resistance of Staphylococcus strains.

Strain	Resistance phenotype <sup>a</sup>	Resistance genotype			
•		Aminoglycosides	ß-lactams	Macrolides	Efflux pumps
S. aureus ATCC 29213	PEN	-	blaZ, blaIShaemolyt	-	msrA, mreA
S. aureus P2116	PEN, Ei, DAi,	-	blaZ, blaIShaemolyt	-	msrA, mreA
S. aureus C5010	TOB, PEN, OXA, E, DA	aadD	blaZ, blaIShaemolyt, mecA	ermA	msrA, mreA
S. aureus MW2	PEN, OXA, Trimethoprim	-		-	msrA, mreA
S. epidermidis ATCC 12228	PEN	-	blaZ, blaIShaemolyt	-	-
S. epidermidis BC1920	GEN, TOB, PEN, OXA, E, DA	aadD, aacA- aphD, aacA_aphDStwar	blaZ, blaIShaemolyt, mecA	ermC	-
S. haemolyticus DSM 20263	susceptible	-	-	-	-
S. hominis DSM 20228	susceptible	-	-	-	-
S. lugdunensis DSM 4804	susceptible	-	-	-	-
S. saprophyticus ATCC 14953	susceptible	-	-	-	-
S. warneri DSM 20316	susceptible	-	-	-	-

<sup>&</sup>lt;sup>a</sup>PEN, penicillin; OXA, oxacillin; DA, clindamycin; E, erythromycin; TOB, tobramycin; GEN, gentamicin; i, intermediary resistance.

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Resistance to macrolides (erythromycin and clindamycin) was conferred by the ermA gene to the clinical MRSA isolate C5010 and by ermC to the MRSE isolate

<sup>&</sup>lt;sup>b</sup>Relative low fluorescence intensity (Median fluorescence – background <18.000).

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BC1920. Both strains also showed resistance to tobramycin, which was conferred by the *aadD* gene, additionally the *S. epidermidis* isolate was resistant to gentamycin, due to posession of the *aacA-aphD* gene (Table 12). With the exception of the *S. epidermidis* strains, all CoNS showed a susceptible phenotype and did not hybridize with any of the resistance gene probes.

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Example 2.14: Strain discrimination and detection of virulence genes in S. aureus Virulence gene probes, showing varying fluorescence intensities after hybridization with DNA of four different *S. aureus* strains are listed in Table 13.

<u>Tab. 13:</u> Hybridization of *S. aureus* virulence gene probes: -, Median fluorescence <10000; +, Median fluorescence  $\geq$ 10000-20000; ++, Median fluorescence >20000-50000; +++, Median fluorescence <50000. Percentage of identity for gene probe sequences complementary to the genes present in the fully sequenced strain MW2 is given in the last column.

S. aureus	S. arueus	S. aureus	MRSA	MRSA	Sequence	
virulence	ATCC	P2116	C5010	MW2	identity with	
gene probes	29213				MW2	
					genome	
					sequence	
epiP-bsaP	_	-	-	+++	100%	
hsdS1	+++	-	+++	<u>.</u>	Not present	
SAV0441	+++	-	+++	+	Not present	
bsaE	-	-	+	+++	100%	
bsaG	++	++	+++	+++	100%	
cap5	+++	-	+++	-	Not present	
сар8	-	+++	-	+++	100%	
EDIN	+++	-	-	<u></u>	Not present -	
lukF	+	++	++	+++	95%	
lukS1	+	+	++	+++	98%	
sea	+++	-	+++	+++	100%	
sec1	-	-	+	+++	98%	
seg1	+++	_	+++	+	Not present	

seh	_	+	++	+++	100%
sel	-	_	+	+++	99%

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For other S. aureus gene probes the fluorescence intensities were either very low (MF-B <10000) for all four strains indicating the absence of the according gene (eg. tst, eta or etb) or very high (MF-B >50000), indicating the presence of the according gene in all four strains (eg. hglA, hglB, hglC, NAG, sak, set, sprV8). Capsular polysaccharides enhance microbial virulence by rendering the bacterium resistant to phagocytosis. Among the eleven capsular serotypes of S. aureus, serotypes 5 and 8 account for ≈25% and 50%, respectively, of isolates recovered from humans. Moreover, these two serotypes, carrying the genes cap5 and cap8, are prevalent among isolates from clinical infections as well as from commensal sources. By microarray hybridization the cap5 gene was detected in the ATCC 29213 strain and the clinical MRSA isolate C5010, while cap8 was detected in the clinical isolate P2116 and the community-aquired MRSA strain MW2 (Table 13). The latter strain hybridized to many virulence gene probes including the leukocidin gene probes lukF and lukS and the enterotoxin gene probes sea, sec, seh and sel. This microarray gene profile is in perfect concordance with genome sequence of this fully sequenced strain, which produces the Panton-Valentine leukocidin (PVL), encoded by lukF and lukS. Panton-Valentine leukocidin forms non-specific pores in leukocyte plasma membranes, which result in increased permeability and eventual host cell lysis. While strain MW2 does not harbor the gene seg encoding enterotoxin G, this gene was detected in the ATCC strain and the clinical MRSA isolate C5010, which both also showed hybridization with sea (Enterotoxin A). In contrast, the clinical isolate P2116 showed no or only minor hybridization with these virulence probes. From these results it can be concluded that microarray hybridization patterns allow the discrimination of different S. aureus strains as well as the detection of clinically relevant virulence determinants.

Example 2.15: Strain discrimination and detection of virulence genes in *E. coli* Virulence gene probes, showing varying fluorescence intensities after hybridization with DNA of seven different *E. coli* strains are listed in Table 14.

<u>Tab. 14:</u> Hybridization of *E. coli* virulence gene probes: -, Median fluorescence <10000; +, Median fluorescence  $\geq$ 10000 -20000; ++, Median fluorescence  $\geq$ 20000-50000; +++, Median fluorescence  $\leq$ 50000.

	E. coli	E. coli	E. coli				
	CIP	ATCC	CIP	CIP	U10338-1	U10164-2	U10248-1
	105893	25922	81.88	74.14	ESBL	ESBL,	GEN-R
	ESBL					GEN-R	
<i>b</i> 1169	+++	++	+++	++	+++	+++	-
ycdS	+++	++ .	+++	++	+++	+++	<del>-</del>
ymcA	+++	+	+++	_	-	+	+
b1202	+++	-	+++	-	-	-	+++
fteA	+	+	-	++	+++	+++	++
iucA	+	++	-	_	+++	+++	+++
iucB	_	++		-	++	+++	++
iucC	+	++	-	-	+++	+++	+++
papG	_	+++	_	++	· <b>-</b>	-	+++

None of the listed genes was detected in all seven strains. Major hybridization of the iuc aerobactin synthesis genes was detected for four strains. The genes fteA (allele of papA) and papG, both involved in adhesion to host cells and virulence in urinary tract infections were detected in five strains. The three clinical isolates U10338-1, U10164-2 and U10248-1 were all isolated-from patients with urinary tract infections. Based on the virulence hybridization pattern, strains U10338-1 and U10164-2 are nearly identical, while strain U10248-1 can be clearly discriminated.

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### **Sequence Listing – Free text**

### a) Probe sequences

SEQ ID NO	Probe name	Template source
1	cataSaur_1_1	Staphylococcus aureus
2	cataSaur_1_2	Staphylococcus aureus
3	clfA_1_1	Staphylococcus aureus
4	clfB_1_1	Staphylococcus aureus
5	coa_1_1	Staphylococcus aureus
6	coa_1_2	Staphylococcus aureus
7	I-clpC_1_1	Staphylococcus aureus
8	I-clpP_1_1	Staphylococcus aureus
9	I-ctaA_1_1	Staphylococcus aureus
10	I-ctsR_1_1	Staphylococcus aureus
11	I-dltA_1_1	Staphylococcus aureus
12	I-dltB_1_1	Staphylococcus aureus
13	I-dltC_1_1	Staphylococcus aureus
14	I-dnaK_1_1	Staphylococcus aureus
15	I-elkT_1_1	Staphylococcus aureus
16	I-femD_1_1	Staphylococcus aureus
17	I-glnA_1_1	Staphylococcus aureus
18	I-glnR_1_1	Staphylococcus aureus
19	I-grlA_1_1	Staphylococcus aureus
20	I-grlB_1_1	Staphylococcus aureus
21	I-groEL_1_1	Staphylococcus aureus
22	I-groES_1_1	Staphylococcus aureus
23	I-hemA_1_1	Staphylococcus aureus
24	I-hemE_1_1	Staphylococcus aureus
25	I-hemH_1_1	Staphylococcus aureus
26	I-hemL_1_1	Staphylococcus aureus
27	I-hemY_1_1	Staphylococcus aureus
28	I-lepA_1_1	Staphylococcus aureus
29	I-lrgA_1_1	Staphylococcus aureus
30	I-lrgB_1_1	Staphylococcus aureus
31	I-lytM_1_1	Staphylococcus aureus
32	I-menB_1_1	Staphylococcus aureus
33	I-menD_1_1	Staphylococcus aureus
34	I-menE_1_1	Staphylococcus aureus
35	I-menF_1_1	Staphylococcus aureus
36	I-mreB_1_1	Staphylococcus aureus
37	I-mreR_1_1	Staphylococcus aureus
38	I-mutL_1_1	Staphylococcus aureus
39	I-mutS_1_1	Staphylococcus aureus
40	I-NAG_1_1	Staphylococcus aureus
41	I-pbg_1_1	Staphylococcus aureus
42	I-pbpF_1_1	Staphylococcus aureus
43	I-pdhB_1_1	Staphylococcus aureus

SEQ ID NO	Probe name	Template source
44	I-pdhC_1_1	Staphylococcus aureus
45	I-rsbU_1_1	Staphylococcus aureus
46	I-rsbV_1_1	Staphylococcus aureus
47	I-rsbW_1_1	Staphylococcus aureus
48	I-sgp_1_1	Staphylococcus aureus
49	I-sirR_1_1	Staphylococcus aureus
50	I-sodA_1_1	Staphylococcus aureus
51	I-sodB_1_1	Staphylococcus aureus
52	I-sstA_1_1	Staphylococcus aureus
53	I-sstB_1_1	Staphylococcus aureus
54	I-sstC_1_1	Staphylococcus aureus
55	I-sstD_1_1	Staphylococcus aureus
56	I-trx_1_1	Staphylococcus aureus
57	I-yhiN_1_1	Staphylococcus aureus
58	epiP-bsaP_1_1	Staphylococcus aureus
59	geh_1_1	Staphylococcus aureus
60	gyrA_1_1	Staphylococcus aureus
61	gyrB_1_1	Staphylococcus aureus
62	hemB_1_1	Staphylococcus aureus
63	hemC_1_1	Staphylococcus aureus
64	hemD_1_1	Staphylococcus aureus
65	hemN_1_1	Staphylococcus aureus
66	hsdS_1_1	Staphylococcus aureus
67	hsdS_2_1	Staphylococcus aureus
68	lip_1_1	Staphylococcus aureus
69	menC_1_1	Staphylococcus aureus
70	murC_1_1	Staphylococcus aureus
71	nuc_1_1	Staphylococcus aureus
72	pdhD_1_1	Staphylococcus aureus
73	rpoB_1_1	Staphylococcus aureus
74	SAV0431_1_1	Staphylococcus aureus
75	SAV0439_1_1	Staphylococcus aureus
76	SAV0440_1_1	Staphylococcus aureus
77	SAV0441_1_1	Staphylococcus aureus
78	sigB_1_1	Staphylococcus aureus
79	spa_1_2	Staphylococcus aureus
80	sstC_1_1	Staphylococcus aureus
81	tag_1_1	Staphylococcus aureus
82	tyrA_1_1	Staphylococcus aureus
83	I-aroC_1_1	Staphylococcus aureus
84	I-aroA_1_1	Staphylococcus aureus
85	I-cna_1_1	Staphylococcus aureus
86	I-ebpS_1_1	Staphylococcus aureus
87	I-eno_1_1	Staphylococcus aureus
88	I-fbpA_1_1	Staphylococcus aureus
89	I-fib_1_1	Staphylococcus aureus

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SEQ ID NO	Probe name	Template source
90	I-fnbB_1_1	Staphylococcus aureus
91	I-srtA_1_1	Staphylococcus aureus
92	I-stpC_1_1	Staphylococcus aureus
93	I-fnbA_1_1	Staphylococcus aureus
94	I-spa_1_1	Staphylococcus aureus
95	I-aroE_1_1	Staphylococcus aureus
96	I-aroF_1_1	Staphylococcus aureus
97	I-aroG_1_1	Staphylococcus aureus
98	I-asp23_1_1	Staphylococcus aureus
99	I-atl_1_1	Staphylococcus aureus
100	bsaE_1_1	Staphylococcus aureus
101	bsaG_1_1	Staphylococcus aureus
102	cap5h_1_1	Staphylococcus aureus
103	cap5i_1_1	Staphylococcus aureus
104	cap5j_1_1	Staphylococcus aureus
105	cap5k_1_1	Staphylococcus aureus
106	cap8H_1_1	Staphylococcus aureus
107	cap8I_1_1	Staphylococcus aureus
108	cap8J_1_1	Staphylococcus aureus
109	cap8K_1_1	Staphylococcus aureus
110	I-hld_1_1	Staphylococcus aureus
111	I-hysA_1_1	Staphylococcus aureus
112	I-IgGbg_1_1	Staphylococcus aureus
113	EDIN_1_1	Staphylococcus aureus
114	eta_1_1	Staphylococcus aureus
115	etb_1_1	Staphylococcus aureus
116	hglA_1_1	Staphylococcus aureus
117	hgIA_2_1	Staphylococcus aureus
118	hglB_1_1	Staphylococcus aureus
119	hgIC_2_1	Staphylococcus aureus
120	hla_1_1	Staphylococcus aureus
121	hlb_1_2	Staphylococcus aureus
122	lukF_1_1	Staphylococcus aureus
123	lukS_1_1	Staphylococcus aureus
124	lukS_2_1	Staphylococcus aureus
125	NAG_1_1	Staphylococcus aureus
126	sak_1_1	Staphylococcus aureus
127	sea_1_1	Staphylococcus aureus
128	seb_1_1	Staphylococcus aureus
129	sec1_1_1	Staphylococcus aureus
130	seg_1_1	Staphylococcus aureus
131	seh_1_1	Staphylococcus aureus
132	sel_1_1	Staphylococcus aureus
133	set15_1_1	Staphylococcus aureus
134	set6_1_1	Staphylococcus aureus
135	set7_1_1	Staphylococcus aureus

SEQ ID NO	Probe name	Template source
136	set8_1_1	Staphylococcus aureus
137	sprV8_1_1	Staphylococcus aureus
138	tst_1_1	Staphylococcus aureus
139	I-sdrC_1_1	Staphylococcus aureus
140	I-sdrD_1_1	Staphylococcus aureus
141	I-sdrE_1_1	Staphylococcus aureus
142	b1169_1_1	Escherichia coli
143	envZ_1_1	Escherichia coli
144	fliCb_1_1	Escherichia coli
145	nfrB_1_1	Escherichia coli
146	nlpA_1_1	Escherichia coli
147	pilAe_1_1	Escherichia coli
148	yacH_1_1	Escherichia coli
149	yagX_1_1	Escherichia coli
150	ycdS_1_1	Escherichia coli
151	yciQ_1_1	Escherichia coli
152	ymcA_1_1	Escherichia coli
153	b1202_1_1	Escherichia coli
154	eae_1_1	Escherichia coli
155	eltB_1_1	Escherichia coli
156	escR_1_1	Escherichia coli
157	escT_1_1	Escherichia coli
158	escU_1_1	Escherichia coli
159	espB_1_1	Escherichia coli
160	fes_1_1	Escherichia coli
161	fes_2_1	Escherichia coli
162	fteA_1_1	Escherichia coli
163	hlyA_1_1	Escherichia coli
164	hlyB_1_1	Escherichia coli
165	iucA_1_1	Escherichia coli
166	iucB_1_1	Escherichia coli
167	iucC_1_1	Escherichia coli
168	papG_1_1	Escherichia coli
169	rfbE_1_1	Escherichia coli
170	shuA_1_1	Escherichia coli
171	SLTII_1_1	Escherichia coli
172	toxA-LTPA_1_1	Escherichia coli
173	VT2vaB_1_1	Escherichia coli
174	ardeSE0106_1_1	Staphylococcus epidermidis
175	ardeSE0107_1_1	Staphylococcus epidermidis
	aroiSE0105_1_1	Staphylococcus epidermidis
177	atlE_1_1	Staphylococcus epidermidis
	agrB_1_1	Staphylococcus epidermidis
179	agrC_1_1	Staphylococcus epidermidis
180	alphSE1368_1_1	Staphylococcus epidermidis
181	gad_1_1	Staphylococcus epidermidis

SEQ ID NO	Probe name	Template source
182	glucSE1191_1_1	Staphylococcus epidermidis
183	hsp10_1_1	Staphylococcus epidermidis
184	icaA_1_1	Staphylococcus epidermidis
185	icaB_1_1	Staphylococcus epidermidis
186	mvaSSepid_1_1	Staphylococcus epidermidis
187	nitreSE1972_1_1	Staphylococcus epidermidis
188	nitreSE1974_1_1	Staphylococcus epidermidis
189	nitreSE1975_1_1	Staphylococcus epidermidis
190	oiamtSE1209_1_1	Staphylococcus epidermidis
191	ORF1Sepid_1_1	Staphylococcus epidermidis
192	ORF3bSepid_1_1	Staphylococcus epidermidis
193	qacR_1_1	Staphylococcus epidermidis
194	sin_1_1	Staphylococcus epidermidis
195	ureSE1861_1_1	Staphylococcus epidermidis
196	ureSE1863_1_1	Staphylococcus epidermidis
197	ureSE1864_1_1	Staphylococcus epidermidis
198	ureSE1865_1_1	Staphylococcus epidermidis
199	ureSE1867_1_1	Staphylococcus epidermidis
200	gcaD_1_1	Staphylococcus epidermidis
201	hld_orf5_1_1	Staphylococcus epidermidis
202	icaC_1_1	Staphylococcus epidermidis
203	icaD_1_1	Staphylococcus epidermidis
204	icaR_1_1	Staphylococcus epidermidis
205	psm_beta1and2_1_1	Staphylococcus epidermidis
206	purR_1_1	Staphylococcus epidermidis
207	spoVG_1_1	Staphylococcus epidermidis
208	yabJ_1_1	Staphylococcus epidermidis
209	folQShaemolyt_1_1	Staphylococcus haemolyticus
210	mvaCShaemolyticus_1_1	Staphylococcus haemolyticus
211	mvaDShaemolyt_1_1	Staphylococcus haemolyticus
	mvaK1Shaemolyticus_1_1	Staphylococcus haemolyticus
213	mvaSShaemolyticus_1_1	Staphylococcus haemolyticus
214	RNApolsigm_1_1	Staphylococcus haemolyticus
215	lipShaemolyt_1_1	Staphylococcus haemolyticus
216	agrB2Stalugd_1_1	Staphylococcus lugdunensis
217	agrC2Stalugd_1_1	Staphylococcus lugdunensis
	agrCStalugd_1_1	Staphylococcus lugdunensis
219	slamStalugd_1_1	Staphylococcus lugdunensis
	fblStalugd_1_1	Staphylococcus lugdunensis
	slushABCStalugd_1_1	Staphylococcus lugdunensis
	RNApolsigmSsapro_1_1	Staphylococcus saprophyticus
223	RNApolsigmSsapro_1_2	Staphylococcus saprophyticus
224	msrw1Stwar_1_1	Staphylococcus warneri
225	nukMStwar_1_1	Staphylococcus warneri
226	proDStwar_1_1	Staphylococcus warneri
227	proMStwar_1_1	Staphylococcus warneri
225 226	nukMStwar_1_1 proDStwar_1_1	Staphylococcus warneri Staphylococcus warneri

SEQ ID	Probe name	Template source
228	sigrpoStwar_1_1	Staphylococcus warneri
229	tnpStwar_1_1	Staphylococcus warneri
230	gehAStwar_1_1	Staphylococcus warneri
231	ARG56_1_1	Candida albicans
232	ASL43f_1_1	Candida albicans
233	BGL2 1 1	Candida albicans
234	CACHS3_1_1	Candida albicans
235	CCT8_1_1	Candida albicans
236	CDC37 1 1	Candida albicans
237	CEF3_1_1	Candida albicans
238	CHS1_1_1	Candida albicans
239	CHS2_1_1	Candida albicans
240	CHS4_1_1	Candida albicans
241	CHS5_1_1	Candida albicans
242	CHT1_1_1	Candida albicans
243	CHT2_1_1	Candida albicans
244	CHT4_1_1	Candida albicans
245	CSA1_1_1	Candida albicans
246	5triphosphatase_1_1	Candida albicans
247	AAF1_1_1	Candida albicans
248	ADH1_1_1	Candida albicans
249	ALS1_1_1	Candida albicans
250	ALS7_1_1	Candida albicans
251	EDT1_1_1	Candida albicans
252	ELF_1_1	Candida albicans
253	ESS1_1_1	Candida albicans
254	FAL1_1_1	Candida albicans
255	GAP1_1_1	Candida albicans
256	GNA1_1_1	Candida albicans
257	GSC1_1_1	Candida albicans
258	GSL1_1_1	Candida albicans
259-	HIS1_1_1	Candida albicans
260	HTS1_1_1	Candida albicans
261	HWP1_2_1	Candida albicans
262	HYR1_1_1	Candida albicans
263	INT1a_1_1	Candida albicans
264	KRE15f_1_1	Candida albicans
265	KRE6_1_1	Candida albicans
266	KRE9_1_1	Candida albicans
267	MIG1_1_1	Candida albicans
268	MLS1_1_1	Candida albicans
269	MP65_1_1	Candida albicans
270	NDE1_1_1	Candida albicans
271	PFK2_1_1	Candida albicans
272	PHR1_1_1	Candida albicans
273	PHR2_1_1	Candida albicans

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SEQ ID NO	Probe name	Template source
274	PHR3_1_1	Candida albicans
275	PRA1_1_1	Candida albicans
276	PRS1_1_1	Candida albicans
277	RBT1_1_1	Candida albicans
278	RBT4_1_1	Candida albicans
279	RHO1_1_1	Candida albicans
280	RNR1_1_1	Candida albicans
281	RPB7_1_1	Candida albicans
282	RPL13_1_1	Candida albicans
283	RVS167_1_1	Candida albicans
284	SHA3_1_1	Candida albicans
285	SKN1_1_1	Candida albicans
286	SRB1_1_1	Candida albicans
287	TCA1_1_1	Candida albicans
288	TRP1_1_1	Candida albicans
289	YAE1_1_1	Candida albicans
290	YRB1_1_1	Candida albicans
291	YST1exon2_1_1	Candida albicans
292	CCN1_1_1	Candida albicans
293	CDC28_1_1	Candida albicans
294	CLN2_1_1	Candida albicans
295	CPH1_1_1	Candida albicans
296	CYB1_1_1	Candida albicans
297	EFG1_1_1	Candida albicans
298	MNT1_1_1	Candida albicans
299	RBF1_1_1	Candida albicans
300	RBF1_2_1	Candida albicans
301	RIM101_1_1	Candida albicans
302	RIM8_1_1	Candida albicans
303	SEC14_1_1	Candida albicans
304	SEC4_1_1	Candida albicans
305	TUP1_1_1 <sup>-</sup>	Candida albicans
306	YPT1_1_1	Candida albicans
307	ZNF1CZF1_2_1	Candida albicans
308	arcA_1_1	Enterococcus faecalis
309	arcC_1_1	Enterococcus faecalis
310	bkdA_1_1	Enterococcus faecalis
311	cad_1_1	Enterococcus faecalis
312	camE1_1_1	Enterococcus faecalis
313	csrA_1_1	Enterococcus faecalis
314	dacA_1_1	Enterococcus faecalis
315	dfr_1_1	Enterococcus faecalis
316	dhoD1a_1_1	Enterococcus faecalis
317	ABC-eltA_1_1	Enterococcus faecalis
318	agrBfs_1_1	Enterococcus faecalis
	agrCfs_1_1	Enterococcus faecalis

SEQ ID NO	Probe name	Template source
320	dnaE_1_1	Enterococcus faecalis
321	ebsA_1_1	Enterococcus faecalis
322	ebsB_1_1	Enterococcus faecalis
323	eep_1_1	Enterococcus faecalis
324	efaR_1_1	Enterococcus faecalis
325	gls24_glsB_1_1	Enterococcus faecalis
326	gph_1_1	Enterococcus faecalis
327	gyrAEf_1_1	Enterococcus faecalis
328	metEf_1_1	Enterococcus faecalis
329	mntHCb2_1_1	Enterococcus faecalis
330	mob2_1_1	Enterococcus faecalis
331	mvaD_1_1	Enterococcus faecalis
332	mvaE_1_1	Enterococcus faecalis
333	parC_1_1	Enterococcus faecalis
334	pcfG_1_1	Enterococcus faecalis
335	phoZ_1_1	Enterococcus faecalis
336	polC_1_1	Enterococcus faecalis
337	ptb_1_1	Enterococcus faecalis
338	recS1_1_1	Enterococcus faecalis
339	rpoN_1_1	Enterococcus faecalis
340	tms_1_1	Enterococcus faecalis
341	tyrDC_1_1	Enterococcus faecalis
342	tyrS_1_1	Enterococcus faecalis
343	asa1_1_1	Enterococcus faecalis
344	asp1_1_1	Enterococcus faecalis
345	cgh_1_1	Enterococcus faecalis
346	cylA_1_1	Enterococcus faecalis
347	cylB_1_1	Enterococcus faecalis
348	cylI_1_1	Enterococcus faecalis
349	cylL_cylS_1_1	Enterococcus faecalis
350	cylM_1_1	Enterococcus faecalis
351	ace_1_1	Enterococcus faecalis
352	ef00108_1_1	Enterococcus faecalis
353	ef00109_1_1	Enterococcus faecalis
354	ef0011_1_1	Enterococcus faecalis
355	ef00113_1_1	Enterococcus faecalis
356	ef0012_1_1	Enterococcus faecalis
357	ef0022_1_1	Enterococcus faecalis
358	ef0031_1_1	Enterococcus faecalis
359	ef0032_1_1	Enterococcus faecalis
360	ef0040_1_1	Enterococcus faecalis
361	ef0058_1_1	Enterococcus faecalis
362	eniA_1_1	Enterococcus faecalis
	esa_1_1	Enterococcus faecalis
364	esp_1_1	Enterococcus faecalis
365	gelE_1_1	Enterococcus faecalis

SEQ ID NO	Probe name	Template source
366	groEL_1_1	Enterococcus faecalis
367	groES_1_1	Enterococcus faecalis
368	rt1_1_1	Enterococcus faecalis
369	sala_1_1	Enterococcus faecalis
370	salb_1_1	Enterococcus faecalis
371	sea1_1_1	Enterococcus faecalis
372	sep1_1_1	Enterococcus faecalis
373	vicK_1_1	Enterococcus faecalis
374	yycH_1_1	Enterococcus faecalis
375	yycI_1_1	Enterococcus faecalis
376	yycJ_1_1	Enterococcus faecalis
377	bglB_1_1	Enterococcus faecium
378	bgIR_1_1	Enterococcus faecium
379	bglS_1_1	Enterococcus faecium
380	efmA_1_1	Enterococcus faecium
381	efmB_1_1	Enterococcus faecium
382	efmC_1_1	Enterococcus faecium
383	mreC_1_1	Enterococcus faecium
384	mreD_1_1	Enterococcus faecium
385	mvaDEfaecium_1_1	Enterococcus faecium
386	mvaEEfaecium_1_1	Enterococcus faecium
387	mvaK1Efaecium_1_1	Enterococcus faecium
388	mvaK2Efaecium_1_1	Enterococcus faecium
389	mvaSEfaecium_1_1	Enterococcus faecium
390	orf3_4Efaeciumb_1_1	Enterococcus faecium
391	orf6_7Efaecium_1_1	Enterococcus faecium
392	orf7_8Efaecium_1_1	Enterococcus faecium
393	orf9_10Efaecium_1_1	Enterococcus faecium
394	entA_entI_1_1	Enterococcus faecium
395	entD_1_1	Enterococcus faecium
396	entR_1_1	Enterococcus faecium
397	oep_1_1	Enterococcus faecium
398	sagA_1_2	Enterococcus faecium
399	atsA_1_1	Klebsiella pneumoniae
400	atsB_1_1	Klebsiella pneumoniae
401	budC_1_1	Klebsiella pneumoniae
402	citA_1_1	Klebsiella pneumoniae
403	citW_1_1	Klebsiella pneumoniae
404	citX_1_1	Klebsiella pneumoniae
405	dalD_1_1	Klebsiella pneumoniae
406	dalK_1_1	Klebsiella pneumoniae
407 ·	dalT_1_1	Klebsiella pneumoniae
408	acoA_1_1	Klebsiella pneumoniae
409	acoB_1_1	Klebsiella pneumoniae
410	acoC_1_1	Klebsiella pneumoniae

Klebsiella pneumoniae

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SEQ ID NO	Probe name	Template source
412	fimK_1_1	Klebsiella pneumoniae
413	glfKPN2_1_1	Klebsiella pneumoniae
414	ltrA_1_1	Klebsiella pneumoniae
415	mdcC_1_1	Klebsiella pneumoniae
416	mdcF_1_1	Klebsiella pneumoniae
417	mdcH_1_1	Klebsiella pneumoniae
418	mrkA_1_1	Klebsiella pneumoniae
419	mtrK_1_1	Klebsiella pneumoniae
420	nifF_1_1	Klebsiella pneumoniae
421	nifK_1_1	Klebsiella pneumoniae
422	nifN_1_1	Klebsiella pneumoniae
423	tyrP_1_1	Klebsiella pneumoniae
424	ureA_1_1	Klebsiella pneumoniae
425	wbbO_1_1	Klebsiella pneumoniae
426	wza_1_1	Klebsiella pneumoniae
427	wzb_1_1	Klebsiella pneumoniae
428	wzmKPN2_1_1	Klebsiella pneumoniae
429	wztKPN2_1_1	Klebsiella pneumoniae
430	yojH_1_1	Klebsiella pneumoniae
431	liac_1_1	Klebsiella pneumoniae
432	cim_1_1	Klebsiella pneumoniae
433	aldA_1_1	Klebsiella pneumoniae
434	aldA_2_1	Klebsiella pneumoniae
435	hemly_1_1	Klebsiella pneumoniae
436	pSL017_1_1	Klebsiella pneumoniae
437	pSL020_1_1	Klebsiella pneumoniae
438	rcsA_1_1	Klebsiella pneumoniae
439	rmlC_1_1	Klebsiella pneumoniae
440	rmlD_1_1	Klebsiella pneumoniae
441	waaG_1_1	Klebsiella pneumoniae
442	wbbD_1_1	Klebsiella pneumoniae
443	wbbM_1_1	Klebsiella pneumoniae
444	wbbN_1_1	Klebsiella pneumoniae
445	wbdA_1_1	Klebsiella pneumoniae
446	wbdC_1_1	Klebsiella pneumoniae
447	wztKpn_1_1	Klebsiella pneumoniae
448	yibD_1_1	Klebsiella pneumoniae
449	cymA_1_1	Klebsiella oxytoca
450	cymD_1_1	Klebsiella oxytoca
451	cymE_1_1	Klebsiella oxytoca
452	cymH_1_1	Klebsiella oxytoca
	cymI_1_1	Klebsiella oxytoca
	cymJ_1_1	Klebsiella oxytoca
	ddrA_1_1	Klebsiella oxytoca
	fdt-1_1_1	Klebsiella oxytoca
457	fdt-2_1_1	Klebsiella oxytoca

SEQ ID NO	Probe name	Template source
458	fdt-3_1_1	Klebsiella oxytoca
459	gatY_1_1	Klebsiella oxytoca
460	hydH_1_1	Klebsiella oxytoca
461	masA_1_1	Klebsiella oxytoca
462	nasA_1_1	Klebsiella oxytoca
463	nasE_1_1	Klebsiella oxytoca
464	nasF_1_1	Klebsiella oxytoca
465	pehX_1_1	Klebsiella oxytoca
466	pelX_1_1	Klebsiella oxytoca
467	tagH_1_1	Klebsiella oxytoca
468	tagK_1_1	Klebsiella oxytoca
469	tagT_1_1	Klebsiella oxytoca
470	glpR_1_1	Pseudomonas aeruginosa
471	lasRb_1_1	Pseudomonas aeruginosa
472	OrfX_1_1	Pseudomonas aeruginosa
473	pa0260_1_1	Pseudomonas aeruginosa
474	pa0572_1_1	Pseudomonas aeruginosa
475	pa0625_1_1	Pseudomonas aeruginosa
476	pa0636_1_1	Pseudomonas aeruginosa
477	pa1046_1_1	Pseudomonas aeruginosa
478	pa1069_1_1	Pseudomonas aeruginosa
479	pa1846_1_1	Pseudomonas aeruginosa
480	pa3866_1_1	Pseudomonas aeruginosa
481	pa4082_1_1	Pseudomonas aeruginosa
482	pilAp_1_1	Pseudomonas aeruginosa
483	PilAp2_1_1	Pseudomonas aeruginosa
484	pilC_1_1	Pseudomonas aeruginosa
485	PstP_1_1	Pseudomonas aeruginosa
486	purK_1_1	Pseudomonas aeruginosa
487	uvrDII_1_1	Pseudomonas aeruginosa
488	vsmI_1_1	Pseudomonas aeruginosa
489	vsmR_1_2	Pseudomonas aeruginosa
490	xcpX_1_1_	Pseudomonas aeruginosa
-	aprA_1_1	Pseudomonas aeruginosa
	aprE_1_1	Pseudomonas aeruginosa
493	ctx_1_2	Pseudomonas aeruginosa
494	algB_1_1	Pseudomonas aeruginosa
	algN_1_1	Pseudomonas aeruginosa
	algR_1_1	Pseudomonas aeruginosa
	ExoS_1_1	Pseudomonas aeruginosa
	fpvA_1_1	Pseudomonas aeruginosa
	lasRa_1_1	Pseudomonas aeruginosa
	lipA_1_1	Pseudomonas aeruginosa
	lipH_1_1	Pseudomonas aeruginosa
502	Orf159_1_2	Pseudomonas aeruginosa
503	Orf252_1_1	Pseudomonas aeruginosa

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SEQ ID NO	Probe name	Template source
504	pchG_1_1	Pseudomonas aeruginosa
505	PhzA_1_1	Pseudomonas aeruginosa
506	PhzB_1_1	Pseudomonas aeruginosa
507	PLC_1_1	Pseudomonas aeruginosa
508	plcN_1_1	Pseudomonas aeruginosa
509	plcR_1_1	Pseudomonas aeruginosa
510	pvdD_1_1	Pseudomonas aeruginosa
511	pvdF_1_2	Pseudomonas aeruginosa
512	pyocinS1_1_1	Pseudomonas aeruginosa
513	pyocinS1im_1_1	Pseudomonas aeruginosa
514	pyocinS2_1_1	Pseudomonas aeruginosa
515	pys2_1_1	Pseudomonas aeruginosa
516	pys2_2_1	Pseudomonas aeruginosa
517	rbf303_1_1	Pseudomonas aeruginosa
518	rhlA_1_1	Pseudomonas aeruginosa
519	rhlB_1_1	Pseudomonas aeruginosa
520	rhlR_1_1	Pseudomonas aeruginosa
521	TnAP41_1_2	Pseudomonas aeruginosa
522	toxA_1_1	Pseudomonas aeruginosa
523	cap1EStrpneu_1_1	Streptococcus pneumoniae
524	cap1FStrpneu_1_1	Streptococcus pneumoniae
525	cap1GStrpneu_1_1	Streptococcus pneumoniae
526	cap3AStrpneu_1_1	Streptococcus pneumoniae
527	cap3BStrpneu_1_1	Streptococcus pneumoniae
528	celAStrpneu_1_1	Streptococcus pneumoniae
529	celBStrpneu_1_1	Streptococcus pneumoniae
530	cglAStrpneu_1_1	Streptococcus pneumoniae
531	cglBStrpneu_1_1	Streptococcus pneumoniae
532	cglCStrpneu_1_1	Streptococcus pneumoniae
533	cglDStrpneu_1_1	Streptococcus pneumoniae
534	cinA_1_1	Streptococcus pneumoniae
535	cps14EStrpneum_1_1	Streptococcus pneumoniae
536	cps14FStrpneum_1_1	Streptococcus pneumoniae
537	cps14GStrpneum_1_1	Streptococcus pneumoniae
538	cps14HStrpneum_1_1	Streptococcus pneumoniae
539	cps19aHStrpneum_1_1	Streptococcus pneumoniae
540	cps19aIStrpneum_1_1	Streptococcus pneumoniae
-	cps19aKStrpneum_1_1	Streptococcus pneumoniae
	cps19fGStrpneum_1_1	Streptococcus pneumoniae
543	cps23fGStrpneum_1_1	Streptococcus pneumoniae
544	dexB_1_1	Streptococcus pneumoniae
	dinF_1_1	Streptococcus pneumoniae
546	1760Strpneu_1_1	Streptococcus pneumoniae
	acyPStrpneu_1_1	Streptococcus pneumoniae
	endAStrpneu_1_1	Streptococcus pneumoniae
549	exoAStrpneu_1_1	Streptococcus pneumoniae

SEQ ID NO	Probe name	Template source
550	exp72_1_1	Streptococcus pneumoniae
551	fnIAStrpneu_1_1	Streptococcus pneumoniae
552	fnlBStrpneu_1_1	Streptococcus pneumoniae
553	fnlCStrpneu_1_1	Streptococcus pneumoniae
554	gct18Strpneum_1_1	Streptococcus pneumoniae
555	hexB1_1_1	Streptococcus pneumoniae
556	hftsHstrpneu_1_1	Streptococcus pneumoniae
557	immunofrag1Strpneu_1_1	Streptococcus pneumoniae
558	immunofrag2Strpneu_2_1	Streptococcus pneumoniae
559	immunofrag3Strpneu_2_1	Streptococcus pneumoniae
560	kdtBStrpneu_1_1	Streptococcus pneumoniae
561	lysAStrpneu_1_1	Streptococcus pneumoniae
562	pcpBStrpneu_1_1	Streptococcus pneumoniae
563	pflCStrpneu_1_1	Streptococcus pneumoniae
564	plpA_1_1	Streptococcus pneumoniae
565	prtA1Strpneu_1_1	Streptococcus pneumoniae
566	pspC1Strpneu_1_1	Streptococcus pneumoniae
567	pspC2_1_1	Streptococcus pneumoniae
568	purRStrpneu_1_1	Streptococcus pneumoniae
569	pyrDAStrpneum_1_1	Streptococcus pneumoniae
570	SP0828Strpneu_1_1	Streptococcus pneumoniae
571	SP0830Strpneu_1_1	Streptococcus pneumoniae
572	SP0833Strpneu_1_1	Streptococcus pneumoniae
573	SP0837_38Strpneu_1_1	Streptococcus pneumoniae
574	SP0839Strpneu_1_1	Streptococcus pneumoniae
575	ugdStrpneu_1_1	Streptococcus pneumoniae
576	uncC_1_1	Streptococcus pneumoniae
577	vicXStrepneu_1_1	Streptococcus pneumoniae
578	wchA6bStrpneum_1_1	Streptococcus pneumoniae
579	wci4Strpneum_1_1	Streptococcus pneumoniae
580	wciK4Strpneum_1_1	Streptococcus pneumoniae
581	wciL4Strpneum_1_1	Streptococcus pneumoniae
582	wciN6bStrpneum_1_1	Streptococcus pneumoniae
583	wciO6bStrpneum_1_1	Streptococcus pneumoniae
584	wciP6bStrpneum_1_1	Streptococcus pneumoniae
585	wciY18Strpneum_1_1	Streptococcus pneumoniae
586	wzdbStrpneum_1_1	Streptococcus pneumoniae
587	wze6bStrpneum_1_1	Streptococcus pneumoniae
588	wzy18Strpneum_1_1	Streptococcus pneumoniae
589	wzy4Strpneum_1_1	Streptococcus pneumoniae
590	wzy6bStrpneum_1_1	Streptococcus pneumoniae
591	xpt_1_1	Streptococcus pneumoniae
592	igaStrpneu_1_1	Streptococcus pneumoniae
	lytA_1_1	Streptococcus pneumoniae
594	nanA_1_1	Streptococcus pneumoniae
595	nanBStrpneu_1_1	Streptococcus pneumoniae

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SEQ ID NO	Probe name	Template source
596	pcpCStrpneu_1_1	Streptococcus pneumoniae
597	ply_1_1	Streptococcus pneumoniae
598	prtAStrpneu_1_1	Streptococcus pneumoniae
599	pspA_1_2	Streptococcus pneumoniae
600	SP0834Strpneu_1_1	Streptococcus pneumoniae
601	SP0834Strpneu_1_2	Streptococcus pneumoniae
602	sphtraStrpneu_1_1	Streptococcus pneumoniae
603	wciJStrpneu_1_1	Streptococcus pneumoniae
604	wziyStrpneu_1_1	Streptococcus pneumoniae
605	wzxStrpneu_1_1	Streptococcus pneumoniae
606	cpsA1Strgal_1_1	Streptococcus agalactiae
607	cpsB1Strgal_1_1	Streptococcus agalactiae
608	cpsC1Strgal_1_1	Streptococcus agalactiae
609	cpsD1Strgal_1_1	Streptococcus agalactiae
610	cpsE1Strgal_1_1	Streptococcus agalactiae
611	cpsG1Strgal_1_1	Streptococcus agalactiae
612	cpsIStragal_1_1	Streptococcus agalactiae
613	cpsJStragal_1_1	Streptococcus agalactiae
614	cpsKStragal_1_1	Streptococcus agalactiae
615	cpsMStragal_1_1	Streptococcus agalactiae
616	cpsYStragal_1_1	Streptococcus agalactiae
617	cpsYStragal_2_1	Streptococcus agalactiae
618	cylBStraga_1_1	Streptococcus agalactiae
619	cylEStraga_1_1	Streptococcus agalactiae
620	cylFStraga_1_1	Streptococcus agalactiae
621	cylHStraga_1_1	Streptococcus agalactiae
622	cylIStraga_1_1	Streptococcus agalactiae
623	cylJStraga_1_1	Streptococcus agalactiae
624	cylKStraga_1_1	Streptococcus agalactiae
625	0487Straga_1_1	Streptococcus agalactiae
626	0488Straga_1_1	Streptococcus agalactiae
627	0493Straga_1_1	Streptococcus agalactiae
628	0495Straga_1_1	Streptococcus agalactiae
629	0498Straga_1_1	Streptococcus agalactiae
630	0500Straga_1_1	Streptococcus agalactiae
631	0502Straga_1_1	Streptococcus agalactiae
632	0504Straga_1_1	Streptococcus agalactiae
633	folDStraga_1_1	Streptococcus agalactiae
634	neuA1Strgal_1_1	Streptococcus agalactiae
635	neuB1Strgal_1_1	Streptococcus agalactiae
636	neuC1Strgal_1_1	Streptococcus agalactiae
637	neuD1Strgal_1_1	Streptococcus agalactiae
638	recNStraga_1_1	Streptococcus agalactiae
639	ileSStraga_1_1	Streptococcus agalactiae
640	CAMPfactor_1_1	Streptococcus agalactiae
641	CAMPfactor_2_1	Streptococcus agalactiae
		jet. epitococcus uguiactiue

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SEQ ID NO	Probe name	Template source
642	0499Straga_1_1	Streptococcus agalactiae
643	hylStragal_1_1	Streptococcus agalactiae
644	lipStragal_1_1	Streptococcus agalactiae
645	cyclStrpyog_1_1	Streptococcus pyogenes
646	fah_rph_hlo_Strpyog_1_1	Streptococcus pyogenes
647	int_1_1	Streptococcus pyogenes
648	int315.5_1_1	Streptococcus pyogenes
649	murEStrpyog_1_1	Streptococcus pyogenes
650	oppA_1_1	Streptococcus pyogenes
651	oppCStrpyog_1_1	Streptococcus pyogenes
652	oppD_1_1	Streptococcus pyogenes
653	SPy0382Strpyog_1_1	Streptococcus pyogenes
654	SPy0390Strpyog_1_1	Streptococcus pyogenes
655	SpyM3_1351_1_1	Streptococcus pyogenes
656	vicXStrpyog_1_1	Streptococcus pyogenes
657	DNaseIStrpyog_1_1	Streptococcus pyogenes
658	fba2Strpyog_1_1	Streptococcus pyogenes
659	fhuAStrpyog_1_1	Streptococcus pyogenes
660	fhuB1Strpyog_1_1	Streptococcus pyogenes
661	fhuDStrpyog_1_1	Streptococcus pyogenes
662	fhuGStrpyog_1_1	Streptococcus pyogenes
663	hylA_1_1	Streptococcus pyogenes
664	hylP_1_1	Streptococcus pyogenes
665	hylp2_1_1	Streptococcus pyogenes
666	oppB_1_1	Streptococcus pyogenes
667	ropB_1_1	Streptococcus pyogenes
668	scpAStrpyog_1_1	Streptococcus pyogenes
669	sloStrpyog_1_1	Streptococcus pyogenes
670	smez-4Strpyog_1_1	Streptococcus pyogenes
671	sof_1_1	Streptococcus pyogenes
672	sof_2_1	Streptococcus pyogenes
673	speA_1_1	Streptococcus pyogenes
674	speB2Strpyog_1_1	Streptococcus pyogenes
675	speCStrpyog_1_1	Streptococcus pyogenes
676	speJStrpyog_1_1	Streptococcus pyogenes
677	srtBStrpyog_1_1	Streptococcus pyogenes
678	srtCStrpyog_1_1	Streptococcus pyogenes
679	srtEStrpyog_1_1	Streptococcus pyogenes
680	srtFStrpyog_1_1	Streptococcus pyogenes
681	srtGStrpyog_1_1	Streptococcus pyogenes
682	srtIStrpyog_1_1	Streptococcus pyogenes
683	srtKStrpyog_1_1	Streptococcus pyogenes
684	srtRStrpyog_1_1	Streptococcus pyogenes
685	srtTStrpyog_1_1	Streptococcus pyogenes
686	vicKStrpyog_1_1	Streptococcus pyogenes
687	573Stprmut_1_1	Streptococcus viridans

SEQ ID NO	Probe name	Template source
688	580SStprmut_1_1	Streptococcus viridans
689	581_582SStprmut_1_1	Streptococcus viridans
690	584SStprmut_1_1	Streptococcus viridans
691	dltAStrmut_1_1	Streptococcus viridans
692	dltBStrmut_1_1	Streptococcus viridans
693	dltCppx1Strmut_1_1	Streptococcus viridans
694	dltDStrmut_1_1	Streptococcus viridans
695	lichStrbov_1_1	Streptococcus viridans
696	lytRStprmut_1_1	Streptococcus viridans
697	lytSStprmut_1_1	Streptococcus viridans
698	pepQStrrmut_1_1	Streptococcus viridans
699	pflCStrmut_1_1	Streptococcus viridans
700	recNStprmut_1_1	Streptococcus viridans
701	ytqBStrmut_1_1	Streptococcus viridans
702	hlyXStrmut_1_1	Streptococcus viridans
703	igaStrmitis_1_1	Streptococcus viridans
704	igaStrsanguis_1_1	Streptococcus viridans
705	perMStrmut_1_1	Streptococcus viridans
706	atfA_1_1	Proteus mirabilis
707	atfB_1_1	Proteus mirabilis
708	atfC_1_1	Proteus mirabilis
709	ccmPrmi1_1_1	Proteus mirabilis
710	cyaPrmi_1_1	Proteus mirabilis
711	aad_1_1	Proteus mirabilis
712	flfB_1_1	Proteus mirabilis
713	flfD_1_1	Proteus mirabilis
714	flfN_1_1	Proteus mirabilis
715	flhD_1_1	Proteus mirabilis
716	floA_1_1	Proteus mirabilis
717	ftsK_1_1	Proteus mirabilis
718	gstB_1_1	Proteus mirabilis
719	hemCPrmi_1_1	Proteus mirabilis
720	hemDPrmi_1_1	Proteus mirabilis
721	hev_1_1	Proteus mirabilis
722	katA_1_1	Proteus mirabilis
723	lpp1_1_1	Proteus mirabilis
724	menE_1_1	Proteus mirabilis
725	mfd_1_1	Proteus mirabilis
726	nrpA_1_1	Proteus mirabilis
727	nrpB_1_1	Proteus mirabilis
728	nrpG_1_1	Proteus mirabilis
729	nrpS_1_1	Proteus mirabilis
730	nrpT_1_1	Proteus mirabilis
731	nrpU_1_1	Proteus mirabilis
732	pat_1_1	Proteus mirabilis
733	pmfA_1_1	Proteus mirabilis

SEQ ID NO	Probe name	Template source
734	pmfC_1_1	Proteus mirabilis
735	pmfE_1_1	Proteus mirabilis
736	ppaA_1_1	Proteus mirabilis
737	rsbA_1_1	Proteus mirabilis
738	rsbC_1_1	Proteus mirabilis
739	speB_1_1	Proteus mirabilis
740	stmA_1_1	Proteus mirabilis
741	stmB_1_1	Proteus mirabilis
742	terA_1_1	Proteus mirabilis
743	terD_1_1	Proteus mirabilis
744	umoA_1_1	Proteus mirabilis
745	umoB_1_1	Proteus mirabilis
746	umoC_1_1	Proteus mirabilis
747	ureR_1_1	Proteus mirabilis
748	xerC_1_1	Proteus mirabilis
749	ygbA_1_1	Proteus mirabilis
750	flaA_1_1	Proteus mirabilis
751	flaD_1_1	Proteus mirabilis
752	fliA_1_1	Proteus mirabilis
753	hpmA_1_1	Proteus mirabilis
754	hpmB_1_1	Proteus mirabilis
755	lpsPrmi_1_1	Proteus mirabilis
756	mrpA_1_1	Proteus mirabilis
757	mrpB_1_1	Proteus mirabilis
758	mrpC_1_1	Proteus mirabilis
759	mrpD_1_1	Proteus mirabilis
760	mrpE_1_1	Proteus mirabilis
761	mrpF_1_1	Proteus mirabilis
762	mrpG_1_1	Proteus mirabilis
763	mrpH_1_1	Proteus mirabilis
764	mrpI_1_1	Proteus mirabilis
765	mrpJ_1_1	Proteus mirabilis
766	patA_1_1	Proteus mirabilis
767	putA_1_1	Proteus mirabilis
768	uca_1_1	Proteus mirabilis
769	ureDPrmi_1_1	Proteus mirabilis
770	ureEPrmi_1_1	Proteus mirabilis
771	ureFPrmi_1_1	Proteus mirabilis
772	zapA_1_1	Proteus mirabilis
773	zapB_1_1	Proteus mirabilis
774	zapD_1_1	Proteus mirabilis
775	zapE_1_1	Proteus mirabilis
776	envZPrvu_1_1	Proteus vulgaris
777	frdC_1_1	Proteus vulgaris
778	frdD_1_1	Proteus vulgaris
779	infBPrvu_1_1	Proteus vulgaris
		j occus vargaris

SEQ ID NO	Probe name	Template source
780	lad_1_1	Proteus vulgaris
781	tna2_1_1	Proteus vulgaris
782	end_1_1	Proteus vulgaris
783	pqrA_1_1	Proteus vulgaris
784	urg_1_1	Proteus vulgaris
785	blaIMP-7_1_1	Pseudomonas aeruginosa
786	mecISepid_1_1	Staphylococcus epidermidis
787	blaOXA-10_1_2	Pseudomonas aeruginosa
788	blaB_1_1	Proteus vulgaris
789	ampC_1_1	Klebsiella oxytoca
790	I-blaR_1_1	Staphylococcus aureus
791	blaOXA-32_1_1	Pseudomonas aeruginosa
792	bla-CTX-M-22_1_1	Klebsiella pneumoniae
793	pbp2aStrpneu_1_1	Streptococcus pneumoniae
794	blaSHV-1_1_1	Klebsiella pneumoniae
795	blaOXA-2_1_1	Salmonella typhimurium
796	blaRShaemolyt_1_1	Staphylococcus haemolyticus
797	blaIMP-7_1_2	Pseudomonas aeruginosa
798	I-mecR_1_1	Staphylococcus aureus
799	blaOXY_1_1	Klebsiella oxytoca
800	dacCStrpyog_1_1	Streptococcus pyogenes
801	femA_1_1	Staphylococcus aureus
802	mecA_1_1	Staphylococcus aureus
803	blaIShaemolyt_1_1	Staphylococcus haemolyticus
804	blavim_1_1	Pseudomonas aeruginosa
805	pbp2b_1_1	Streptococcus pneumoniae
806	pbp2primeSepid_1_1	Staphylococcus epidermidis
807	pbp2x_1_1	Streptococcus pneumoniae
808	pbp3Saureuc_1_1	Staphylococcus aureus
809	pbp4_1_1	Enterococcus faecalis
810	pbp5Efaecium_1_1	Enterococcus faecium
811	pbpC_1_1	Enterococcus faecalis
812	I-mecI_1_1	Staphylococcus aureus
813	pbp1a_1_1	Streptococcus pneumoniae
814	I-blaI_1_1	Staphylococcus aureus
815	blaTEM-106_1_1	Escherichia coli
816	blaOXY-KLOX_1_1	Klebsiella oxytoca
817	ftsWEF_1_1	Enterococcus faecium
818	fmhB_1_1	Staphylococcus aureus
819	cumA_1_1	Proteus vulgaris
	femBShaemolyt_1_1	Staphylococcus haemolyticus
	blaPER-1_1_1	Pseudomonas aeruginosa
	bla_FOX-3_1_1	Klebsiella oxytoca
	blaA_1_1	Proteus vulgaris
824	psrb_1_1	Enterococcus faecium
825	fmhA_1_1	Staphylococcus aureus

SEQ ID NO	Probe name	Template source
826	mecR1Sepid_1_1	Staphylococcus epidermidis
827	blaZ_1_1	Staphylococcus aureus
828	blaOXA-1_1_1	Plasmid RGN238
829	fox-6_1_1	Klebsiella pneumoniae
830	blaPrmi_1_1	Proteus mirabilis
831	aacA_aphDStwar_1_1	Staphylococcus warneri
832	aacC1_1_2	Pseudomonas aeruginosa
833	aacC2_1_1	Escherichia coli
834	strB_1_1	Escherichia coli
835	aadA_1_1	Enterococcus faecalis
836	aadB_1_2	Escherichia coli
837	aadD_1_1	Staphylococcus aureus
838	aacA4_1_2	Pseudomonas aeruginosa
839	strA_1_1	Escherichia coli
840	aph-A3_1_1	Staphylococcus aureus
841	aacC1_1_1	Pseudomonas aeruginosa
842	aacA4_1_1	Pseudomonas aeruginosa
843	aacA-aphD_1_1	Staphylococcus aureus
844	I-spc_1_1	Staphylococcus aureus
845	aphA3_1_1	synthetic construct
846	ermC_1_1	Staphylococcus aureus
847	linB_1_1	Enterococcus faecium
848	satSA_1_1	Staphylococcus aureus
849	mdrSA_1_1	Staphylococcus aureus
850	I-linA_1_1	Staphylococcus aureus
851	ermB_1_2	Staphylococcus aureus
852	ermA_1_1	Staphylococcus aureus
853	satA_1_1	Enterococcus faecium
854	msrA_1_1	Staphylococcus aureus
855	mphBM_1_1	Staphylococcus aureus
856	mefA_1_1	Streptococcus pyogenes
857	mrx_1_1	Escherichia coli
858	dfrStrpneu_1_1	Streptococcus pneumoniae
859	dfrA_1_1	Staphylococcus aureus
860	cmlA5_1_1	Escherichia coli
861	catEfaecium_1_1	Enterococcus faecium
862	cat_1_1	Staphylococcus aureus
863	tetAJ_1_1	Proteus mirabilis
864	tetL_1_1	Enterococcus faecalis
865	tetM_1_1	Enterococcus faecalis
866	vanH(tn)_1_1	Enterococcus faecium
867	vanA_1_1	Enterococcus faecium
868	vanHB2_1_1	Enterococcus faecium
869	vanR_1_1	Enterococcus faecium
870	vanRB2_1_1	Enterococcus faecium
871	vanS(tn)_1_1	Enterococcus faecium

SEQ ID NO	Probe name	Template source
872	vanSB2_1_1	Enterococcus faecium
873	vanWB2_1_1	Enterococcus faecium
874	ddl_1_1	Enterococcus faecalis
875	ble_1_1	Staphylococcus aureus
876	vanXB2_1_1	Enterococcus faecium
877	vanY(tn)_1_1	Enterococcus faecium
878	vanYB2_1_1	Enterococcus faecium
879	vanB_1_1	Enterococcus faecalis
880	vanZ(tn)_1_1	Enterococcus faecium
881	vanC-2_1_1	Enterococcus flavescens
882	vanX(tn)_1_1	Enterococcus faecium
883	acrB_1_1	Proteus mirabilis
884	mexB_1_2	Pseudomonas aeruginosa
885	I-qacA_1_1	Staphylococcus aureus
886	sulI_1_1	Escherichia coli
887	sul_1_1	Escherichia coli
888	cadBStalugd_1_1	Staphylococcus lugdunensis
889	mexA_1_1	Pseudomonas aeruginosa
890	acrR_1_1	Proteus mirabilis
891	emeA_1_1	Enterococcus faecalis
892	acrA_1_1	Proteus mirabilis
893	rtn_1_1	Proteus vulgaris
894	abcXStrpmut_1_1	Streptococcus mutans
895	qacEdelta1_1_1	Escherichia coli
896	elkT-abcA_1_1	Staphylococcus aureus
897	I-cadA_1_1	Staphylococcus aureus
898	albA_1_1	Klebsiella oxytoca
899	wzm_1_1	Klebsiella pneumoniae
900	msrCb_1_1	Enterococcus faecium
901	nov_1_1	Escherichia coli
902	wzt_1_1	Klebsiella pneumoniae
903	wbbl_1_1	Klebsiella pneumoniae
904	norA23_1_1	Staphylococcus aureus
905	mexR_1_1	Pseudomonas aeruginosa
906	arr2_1_1	Escherichia coli
907	mreA_1_1	Staphylococcus aureus
908	I-cadC_1_1	Staphylococcus aureus
909	uvrA_1_1	Enterococcus faecalis
910	CRD2_1_1	Candida albicans
911	CDR1_1_1	Candida albicans
912	CDR1_2_1	Candida albicans
913	MET3_1_1	Candida albicans
914	FET3_1_1	Candida albicans
915	FTR2_1_1	Candida albicans
916	MDR1-7_1_1	Candida albicans
917	ERG11_1_1	Candida albicans

SEQ ID NO	Probe name	Template source
918	SEC20_1_1	Candida albicans
919	rbcL_1_1	Glycine max
920	LDHA(hu)_1_1	Homo sapiens
921	GAPD(hu)_1_1	Homo sapiens
922	b-Act(hu)_1_1	Homo sapiens
923	ARHGDIA(hu)_1_1	Homo sapiens
924	PGK1(hu)_1_1	Homo sapiens
925	rbcL_1_2	Glycine max
926	16SPa_1_1	Pseudomonas aeruginosa
927	23SEfaecium_2_1	Enterococcus faecium
928	16SStrepyog_1_1	Streptococcus pyogenes
929	16SStrepneu_1_1	Streptococcus pneumoniae
930	16SStrepagalactiae_1_1	Streptococcus agalactiae
931	16SEfaecium_1_1	Enterococcus faecium
932	16SEfaecium_2_1	Enterococcus faecium
933	16SRNAEf_2_1	Enterococcus faecalis
934	16SKpn_1_1	Klebsiella pneumoniae
935	16SSa_3_1	Staphylococcus aureus
936	16SRNAEf_1_1	Enterococcus faecalis
937	16SShominis_1_1	Staphylococcus hominis
938	16SShaemolyt_1_1	Staphylococcus haemolyticus
939	23SEfaecium_1_1	Enterococcus faecium
940	16SrRNAPrmi_1_1	Proteus mirabilis
941	16SrRNAPrvu1_1_1	Proteus vulgaris
942	16SSa_1_1	Staphylococcus aureus
943	16SKlox_1_1	Klebsiella oxytoca
944	p53_1_1	Mus musculus
945	0135mihck_1_1	Dictyostelium discoideum
946	FAN_1_1	Mus musculus
947	0270cap_1_1	Dictyostelium discoideum
2842	16SStrepdysgal_1_1	Streptococcus dysgalactiae
2843	carO_1_1	Acinetobacter baumannii
2844	gacS_1_1	Acinetobacter baumannii
2845	dhbA_1_1	Acinetobacter baumannii
2846	dhbB_1_1	Acinetobacter baumannii
2847	sid_1_1	Acinetobacter baumannii
2848	csuD_1_1	Acinetobacter baumannii
2849	csuC_1_1	Acinetobacter baumannii
2850	tnp-ACIBA_1_1	Acinetobacter baumannii
2851	waaA-ACIBA_1_1	Acinetobacter baumannii
2852	csuB_1_1	Acinetobacter baumannii
2853	csuA_B_1_1	Acinetobacter baumannii
2854	csuA_1_1	Acinetobacter baumannii
2855	put1_1_1	Acinetobacter baumannii
2856	por_1_1	Acinetobacter baumannii
2857	abc_1_1	Acinetobacter baumannii

SEQ ID NO	Probe name	Template source
2858	furACIBA_1_1	Acinetobacter baumannii
2859	dec_1_1	Acinetobacter baumannii
2860	cysI_1_1	Acinetobacter baumannii
2861	trpE_1_1	Acinetobacter baumannii
2862	put3_1_1	Acinetobacter baumannii
2863	ompA-ACIBA_1_1	Acinetobacter baumannii
2864	aacA4ENCL_1_1	Enterobacter cloacae
2865	AdeR-ACIBA_1_1	Acinetobacter baumannii
2866	adeA-ACIBA_1_1	Acinetobacter baumannii
2867	aac(6p)-lb7_1_1	Enterobacter cloacae
2868	adeB-ACIBA_1_1	Acinetobacter baumannii
2869	adeC-ACIBA_1_1	Acinetobacter baumannii
2870	AdeS-ACIBA_1_1	Acinetobacter baumannii
2871	blaL2_1_1	Stenotrophomonas maltophilia
2872	blaMIR-3_1_1	Enterobacter cloacae
2873	ampR_1_1	Enterobacter cloacae
2874	ampC-ENCL_1_1	Enterobacter cloacae
2875	blaL1_1_1	Stenotrophomonas maltophilia
2876	asr_1_1	Enterobacter cloacae
2877	lacZ_1_1	Enterobacter cloacae
2878	ehuS_1_1	Enterobacter cloacae
2879	ehuV_1_1	Enterobacter cloacae
2880	slyA_1_1	Enterobacter cloacae
2881	ORF165_1_1	Enterobacter cloacae
2882	ehuU_1_1	Enterobacter cloacae
2883	ehuT_1_1	Enterobacter cloacae
2884	ORF295_1_1	Enterobacter cloacae
2885	ehuA_1_1	Enterobacter cloacae
2886	ORF400_1_1	Enterobacter cloacae
2887	H+ATPase_1_1	Enterococcus faecium
2888	sulII_1_1	Acinetobacter baumannii
2889	smeE_1_1	Stenotrophomonas maltophilia
2890	eE_1_1	Stenotrophomonas maltophilia
2891	StmPr1_1_1	Stenotrophomonas maltophilia
2892	eD_2_1	Stenotrophomonas maltophilia
2893	ppi_1_1	Stenotrophomonas maltophilia
2894	pmp-STEMA_1_1	Stenotrophomonas maltophilia
2895	pam_1_1	Stenotrophomonas maltophilia
2896	ORF4-STEMA_1_1	Stenotrophomonas maltophilia
2897	ORF2-STEMA_1_1	Stenotrophomonas maltophilia
2898	et_1_1	Stenotrophomonas maltophilia
2899	eF_1_1	Stenotrophomonas maltophilia
2900	StmPr2_1_1	Stenotrophomonas maltophilia
2901	smeF4494_1_1	Stenotrophomonas maltophilia
2902	coa_3_1	Staphylococcus aureus
2903	coa_2_2	Staphylococcus aureus

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SEQ ID NO	Probe name	Template source
2904	fasCAXStrdysg_1_1	Streptococcus dysgalactiae
2905	sloStrep_1_1	Streptococcus dysgalactiae
2906	ydhK_1_1	Staphylococcus hominis
2907	tetA-ACIBA_1_1	Acinetobacter baumannii
2908	tetR-ACIBA_1_1	Acinetobacter baumannii

## b) primer sequences

SEQ ID NO	Probe name	Direction
948	cataSaur_1_1	F(orward)
949	cataSaur_1_1	R(everse)
950	cataSaur_1_2	F
951	cataSaur_1_2	R
952	clfA_1_1	F
953	clfA_1_1	R
954	clfB_1_1	F
955	clfB_1_1	R
956	coa_1_1	F
957	coa_1_1	R
958	coa_1_2	F
959	coa_1_2	R
960	I-clpC_1_1	F
961	I-clpC_1_1	R
962	I-clpP_1_1	F
963	I-clpP_1_1	R
964	I-ctaA_1_1	F
965	I-ctaA_1_1	R
966	I-ctsR_1_1	F
967	I-ctsR_1_1	R
968	I-dltA_1_1	F
969	I-dltA_1_1	R
970	I-dltB_1_1	F
971	I-dltB_1_1	R
972	I-dltC_1_1	F
973	I-dltC_1_1	R
974	I-dnaK_1_1	F
975	I-dnaK_1_1	R
976	I-elkT_1_1	F
977	I-elkT_1_1	R
978	I-femD_1_1	F
979	I-femD_1_1	R
980	I-glnA_1_1	F
981	I-glnA_1_1	R
982	I-glnR_1_1	F

SEQ ID NO	Probe name	Direction
983	I-glnR_1_1	R
984	I-grlA_1_1	F
985	I-grlA_1_1	R
986	I-grlB_1_1	F
987	I-grlB_1_1	R
988	I-groEL_1_1	F
989	I-groEL_1_1	R
990	I-groES_1_1	F
991	I-groES_1_1	R
992	I-hemA_1_1	F
993	I-hemA_1_1	R
994	I-hemE_1_1	F
995	I-hemE_1_1	R
996	I-hemH_1_1	F
997	I-hemH_1_1	R
998	I-hemL_1_1	F
999	I-hemL_1_1	R
1000	I-hemY_1_1	F
1001	I-hemY_1_1	R
1002	I-lepA_1_1	F
1003	I-lepA_1_1	R
1004	I-lrgA_1_1	F
1005	I-lrgA_1_1	R
1006	I-lrgB_1_1	F
1007	I-lrgB_1_1	R
1008	I-lytM_1_1	F
1009	ytm_1_1  I- ytM_1_1	R
1010	I-menB_1_1	F
1011	I-menB_1_1	R
1012	I-menD_1_1	F
1013	I-menD_1_1	'R
1013	I-menE_1_1	F
1014	I-menE_1_1	'R
1016	I-menF_1_1	F
1017	I-menF_1_1	r R
1017	I-mreB_1_1	F
1019	I-mreB_1_1	R
l .		F
1020 1021	I-mreR_1_1	R
1	I-mreR_1_1	F
1022	I-mutL_1_1	ļ.
1023	I-mutL_1_1 I_mutS_1_1	R F
1024	I-mutS_1_1	
1025	I-mutS_1_1	R
1026	I-NAG_1_1	F

SEQ ID NO	Probe name	Direction
1027	I-NAG_1_1	R
1028	I-pbg_1_1	F
1029	I-pbg_1_1	R
1030	I-pbpF_1_1	F
1031	I-pbpF_1_1	R
1032	I-pdhB_1_1	F
1033	I-pdhB_1_1	R
1034	I-pdhC_1_1	F
1035	I-pdhC_1_1	R
1036	I-rsbU_1_1	F
1037	I-rsbU_1_1	R
1038	I-rsbV_1_1	F
1039	I-rsbV_1_1	R
1040	I-rsbW_1_1	F
1041	I-rsbW_1_1	R
1042	I-sgp_1_1	F
1043	I-sgp_1_1	R
1044	I-sirR_1_1	F
1045	I-sirR_1_1	R
1046	I-sodA_1_1	F
1047	I-sodA_1_1	R
1048	I-sodB_1_1	F
1049	I-sodB_1_1	R
1050	I-sstA_1_1	F
1051	I-sstA_1_1	R
1052	I-sstB_1_1	F
1053	I-sstB_1_1	R
1054	I-sstC_1_1	F
1055	I-sstC_1_1	R
1056	I-sstD_1_1	F
1057	I-sstD_1_1	R
1058	I-trx_1_1	F
1059	I-trx_1_1	R
1060	I-yhiN_1_1	F
1061	I-yhiN_1_1	R
1062	epiP-bsaP_1_1	F
1063	epiP-bsaP_1_1	R
1064	geh_1_1	F
1065	geh_1_1	R
1066	gyrA_1_1	F
1067	gyrA_1_1	R
1068	gyrB_1_1	F
1069	gyrB_1_1	R
1070	hemB_1_1	F

SEQ ID NO	Probe name	Direction
1071	hemB_1_1	R
1072	hemC_1_1	F
1073	hemC_1_1	R
1074	hemD_1_1	F
1075	hemD_1_1	R
1076	hemN_1_1	F
1077	hemN_1_1	R
1078	hsdS_1_1	F
1079	hsdS_1_1	R
1080	hsdS_2_1	F
1081	hsdS_2_1	R
1082	lip_1_1	F
1083	lip_1_1	R
1084	menC_1_1	F
1085	menC_1_1	R
1086	murC_1_1	F
1087	murC_1_1	R
1088	nuc_1_1	F
1089	nuc_1_1	R
1090	pdhD_1_1	F
1091	pdhD_1_1	R
1092	rpoB_1_1	F
1093	rpoB_1_1	R
1094	SAV0431_1_1	F
1095	SAV0431_1_1	R
1096	SAV0439_1_1	F
1097	SAV0439_1_1	R
1098	SAV0440_1_1	F
1099	SAV0440_1_1	R
1100	SAV0441_1_1	F
1101	SAV0441_1_1	R
1102	sigB_1_1	F
1103	sigB_1_1	R
1104	spa_1_2	F
1105	spa_1_2	R
1106	sstC_1_1	F
1107	sstC_1_1	R
1108	tag_1_1	F
1109	tag_1_1	R
11.10	tyrA_1_1	F
1111	tyrA_1_1	R
1112	I-aroC_1_1	F
1113	I-aroC_1_1	R
1114	I-aroA_1_1	F

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	T	
SEQ ID NO	Probe name	Direction
1115	I-aroA_1_1	R
1116	I-cna_1_1	F
1117	I-cna_1_1	R
1118	I-ebpS_1_1	F
1119	I-ebpS_1_1	R
1120	I-eno_1_1	F
1121	I-eno_1_1	R
1122	I-fbpA_1_1	F
1123	I-fbpA_1_1	R
1124	I-fib_1_1	F
1125	I-fib_1_1	R
1126	I-fnbB_1_1	F
1127	I-fnbB_1_1	R
1128	I-srtA_1_1	F
1129	I-srtA_1_1	R
1130	I-stpC_1_1	F
1131	I-stpC_1_1	R
1132	I-fnbA_1_1	F
1133	I-fnbA_1_1	R
1134	I-spa_1_1	F
1135	I-spa_1_1	R
1136	I-aroE_1_1	F
1137	I-aroE_1_1	R
1138	I-aroF_1_1	F
1139	I-aroF_1_1	R
1140	I-aroG_1_1	F
1141	I-aroG_1_1	R
1142	I-asp23_1_1	F
1143	I-asp23_1_1	R
1144	I-atl_1_1	F
1145	I-atl_1_1	R
1146	bsaE_1_1	F
1147	bsaE_1_1	R
1148	bsaG_1_1	F
1149		R
1150	cap5h_1_1	F
1151	cap5h_1_1	R
1152	cap5i_1_1	F
1153	cap5i_1_1	R
1154	cap5j_1_1	F
1155	cap5j_1_1	R
1156	cap5k_1_1	F
1157		R
1158	cap8H_1_1	F

SEQ ID NO	Probe name	Direction
1159	cap8H_1_1	R
1160	cap8I_1_1	F
1161	cap8I_1_1	R
1162	cap8J_1_1	F
1163	cap8J_1_1	R
1164	cap8K_1_1	F
1165	cap8K_1_1	R
1166	I-hld_1_1	F
1167	I-hld_1_1	R
1168	I-hysA_1_1	F
1169	I-hysA_1_1	R
1170	I-IgGbg_1_1	F
1171	I-IgGbg_1_1	R
1172	EDIN_1_1	F
1173	EDIN_1_1	R
1174	eta_1_1	F
1175	eta_1_1	R
1176	etb_1_1	F
1177	etb_1_1	R
1178	hglA_1_1	F
1179	hgIA_1_1	R
1180	hgIA_2_1	F
1181	hgIA_2_1	R
1182	hglB_1_1	F
1183	hglB_1_1	R
1184	hglC_2_1	F
1185	hglC_2_1	R
1186	hla_1_1	F
1187	hla_1_1	R
1188	hlb_1_2	F
1189	hlb_1_2	R
1190	lukF_1_1	F
1191	lukF_1_1	R
1192	lukS_1_1	F
1193	lukS_1_1	R
1194	lukS_2_1	F
1195	lukS_2_1	R
1196	NAG_1_1	F
1197	NAG_1_1	R
1198	sak_1_1	F
1199	sak_1_1	R
1200	sea_1_1	F
1201	sea_1_1	R
1202	seb_1_1	F

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SEQ ID NO	Probe name	Direction
1203	seb_1_1	R
1204	sec1_1_1	F
1205	sec1_1_1	R
1206	seg_1_1	F
1207	seg_1_1	R
1208	seh_1_1	F
1209	seh_1_1	R
1210	sel_1_1	F
1211	sel_1_1	R
1212	set15_1_1	F
1213	set15_1_1	R
1214	set6_1_1	F
1215	set6_1_1	R
1216	set7_1_1	F
1217	set7_1_1	R
1218	set8_1_1	F
1219	set8_1_1	R
1220	sprV8_1_1	F
1221	sprV8_1_1	R
1222	tst_1_1	F
1223	tst_1_1	R
1224	I-sdrC_1_1	F
1225	I-sdrC_1_1	R
1226	I-sdrD_1_1	F
1227	I-sdrD_1_1	R
1228	I-sdrE_1_1	F
1229	I-sdrE_1_1	R
1230	b1169_1_1	F
1231	b1169_1_1	R
1232	envZ_1_1	F
1233	envZ_1_1	R
1234	fliCb_1_1	F
1235	fliCb_1_1	R
1236	nfrB_1_1	F
1237	nfrB_1_1	R
1238	nlpA_1_1	F
1239	nlpA_1_1	R
1240	pilAe_1_1	F
1241		R
1242		F
1243	yacH_1_1	R
1244		F
1245		R
1246	ycdS_1_1	F

SEQ ID NO	Probe name	Direction
1247	ycdS_1_1	R
1248	yciQ_1_1	F
1249	yciQ_1_1	R
1250	ymcA_1_1	F
1251	ymcA_1_1	R
1252	b1202_1_1	F
1253	b1202_1_1	R
1254	eae_1_1	F
1255	eae_1_1	R
1256	eltB_1_1	F
1257	eltB_1_1	R
1258	escR_1_1	F
1259	escR_1_1	R
1260	escT_1_1	F
1261	escT_1_1	R
1262	escU_1_1	F
1263	escU_1_1	R
1264	espB_1_1	F
1265	espB_1_1	R
1266	fes_1_1	F
1267	fes_1_1	R
1268	fes_2_1	F
1269	fes_2_1	R
1270	fteA_1_1	F
1271	fteA_1_1	R
1272	hlyA_1_1	F
1273	hlyA_1_1	R
1274	hlyB_1_1	F
1275	hlyB_1_1	R
1276	iucA_1_1	F
1277	iucA_1_1	R
1278	iucB_1_1	F
1279	iucB_1_1	R
1280	iucC_1_1	F
1281	iucC_1_1	R
1282	papG_1_1	F
1283	papG_1_1	R
1284	rfbE_1_1	F
1285	rfbE_1_1	R
1286	shuA_1_1	F
1287	shuA_1_1	R
1288	SLTII_1_1	F
1289	SLTII_1_1	R
1290	toxA-LTPA_1_1	F

SEQ ID NO	Probe name	Direction
1291	toxA-LTPA_1_1	R
1292	VT2vaB_1_1	F
1293	VT2vaB_1_1	R
1294	ardeSE0106_1_1	F
1295	ardeSE0106_1_1	R
1296	ardeSE0107_1_1	F
1297	ardeSE0107_1_1	R
1298	aroiSE0105_1_1	F
1299	aroiSE0105_1_1	R
1300	atIE_1_1	F
1301	atIE_1_1	R
1302	agrB_1_1	F
1303	agrB_1_1	R
1304	agrC_1_1	F
1305	agrC_1_1	R
1306	alphSE1368_1_1	F
1307	alphSE1368_1_1	R
1308	gad_1_1	F
1309	gad_1_1	R
1310	glucSE1191_1_1	F
1311	glucSE1191_1_1	R
1312	hsp10_1_1	F
1313	hsp10_1_1	R
1314	icaA_1_1	F
1315	icaA_1_1	R
1316	icaB_1_1	F
1317	icaB_1_1	R
1318	mvaSSepid_1_1	F
1319	mvaSSepid_1_1	R
1320	nitreSE1972_1_1	F
1321	nitreSE1972_1_1	R
1322	nitreSE1974_1_1	F
1323	nitreSE1974_1_1	R
1324	nitreSE1975_1_1	F
1325	nitreSE1975_1_1	R
1326	oiamtSE1209_1_1	F
1327	oiamtSE1209_1_1	R
1328	ORF1Sepid_1_1	F
1329	ORF1Sepid_1_1	R
1330	ORF3bSepid_1_1	F
1331	ORF3bSepid_1_1	R
1332	qacR_1_1	F
1333	qacR_1_1	R
1334	sin_1_1	F

SEQ ID NO	Probe name	Direction
1335	sin_1_1	R
1336	ureSE1861_1_1	F
1337	ureSE1861_1_1	R
1338	ureSE1863_1_1	F
1339	ureSE1863_1_1	R
1340	ureSE1864_1_1	F
1341	ureSE1864_1_1	R
1342	ureSE1865_1_1	F
1343	ureSE1865_1_1	R
1344	ureSE1867_1_1	F
1345	ureSE1867_1_1	R
1346	gcaD_1_1	F.
1347	gcaD_1_1	R
1348	hld_orf5_1_1	F
1349	hld_orf5_1_1	R
1350	icaC_1_1	F
1351	icaC_1_1	R
1352	icaD_1_1	F
1353	icaD_1_1	R
1354	icaR_1_1	F
1355	icaR_1_1	R
1356	psm_beta1and2_1_1	F
1357	psm_beta1and2_1_1	R
1358	purR_1_1	F
1359	purR_1_1	R
1360	spoVG_1_1	F
1361	spoVG_1_1	R
1362	yabJ_1_1	F
1363	yabJ_1_1	R
1364	folQShaemolyt_1_1	F
1365	folQShaemolyt_1_1	R
1366	mvaCShaemolyticus_1_1	F
1367	mvaCShaemolyticus_1_1	R
1368	mvaDShaemolyt_1_1	F
1369	mvaDShaemolyt_1_1	R
1370	mvaK1Shaemolyticus_1_1	F
1371	mvaK1Shaemolyticus_1_1	R
1372	mvaSShaemolyticus_1_1	F
1373	mvaSShaemolyticus_1_1	R
1374	RNApolsigm_1_1	F
1375	RNApolsigm_1_1	R
1376	lipShaemolyt_1_1	F
1377	lipShaemolyt_1_1	R
1378	agrB2Stalugd_1_1	F

SEQ ID NO	Probe name	Direction
1379	agrB2Stalugd_1_1	R
1380	agrC2Stalugd_1_1	F
1381	agrC2Stalugd_1_1	R
1382	agrCStalugd_1_1	F
1383	agrCStalugd_1_1	R
1384	slamStalugd_1_1	F
1385	slamStalugd_1_1	R
1386	fblStalugd_1_1	F
1387	fblStalugd_1_1	R
1388	slushABCStalugd_1_1	F
1389	slushABCStalugd_1_1	R
1390	RNApolsigmSsapro_1_1	F
1391	RNApolsigmSsapro_1_1	R
1392	RNApolsigmSsapro_1_2	F
1393	RNApolsigmSsapro_1_2	R
1394	msrw1Stwar_1_1	F
1395	msrw1Stwar_1_1	R
1396	nukMStwar_1_1	F
1397	nukMStwar_1_1	R
1398	proDStwar_1_1	F
1399	proDStwar_1_1	R
1400	proMStwar_1_1	F
1401	proMStwar_1_1	R
1402	sigrpoStwar_1_1	F
1403	sigrpoStwar_1_1	R
1404	tnpStwar_1_1	F
1405	tnpStwar_1_1	R
1406	gehAStwar_1_1	F
1407	gehAStwar_1_1	R
1408	ARG56_1_1	F
1409	ARG56_1_1	R
1410	ASL43f_1_1	F
1411	ASL43f_1_1	R
1412	BGL2_1_1	F
1413	BGL2_1_1	R
1414	CACHS3_1_1	F
1415	CACHS3_1_1	R
1416	CCT8_1_1	F
1417	CCT8_1_1	R
1418	CDC37_1_1	F
1419	CDC37_1_1	R
1420	CEF3_1_1	F
1421	CEF3_1_1	R
1422	CHS1_1_1	ļ <sub>F</sub>

SEQ ID NO	Probe name	Direction
1423	CHS1_1_1	R
1424	CHS2_1_1	F
1425	CHS2_1_1	R
1426	CHS4_1_1	F
1427	CHS4_1_1	R
1428	CHS5_1_1	F
1429	CHS5_1_1	R
1430	CHT1_1_1	F
1431	CHT1_1_1	R
1432	CHT2_1_1	F
1433	CHT2_1_1	R
1434	CHT4_1_1	F
1435	CHT4_1_1	R
1436	CSA1_1_1	F
1437	CSA1_1_1	R
1438	5triphosphatase_1_1	F
1439	5triphosphatase_1_1	R
1440	AAF1_1_1	F
1441	AAF1_1_1	R
1442	ADH1_1_1	F
1443	ADH1_1_1	R
1444	ALS1_1_1	F
1445	ALS1_1_1	R
1446	ALS7_1_1	F
1447	ALS7_1_1	R
1448	EDT1_1_1	F
1449	EDT1_1_1	R
1450	ELF_1_1	F
1451	ELF_1_1	R
1452	ESS1_1_1	F
1453	ESS1_1_1	R
1454	FAL1_1_1	F
1455	FAL1_1_1	R
1456	GAP1_1_1	F
1457	GAP1_1_1	R
1458	GNA1_1_1	F
1459	GNA1_1_1	R
1460	GSC1_1_1	F
1461	GSC1_1_1	R
1462	GSL1_1_1	F
1463	GSL1_1_1	R
1464	HIS1_1_1	F
1465	HIS1_1_1	R
1466	HTS1_1_1	F

SEQ ID NO	Probe name	Direction
1467	HTS1_1_1	R
1468	HWP1_2_1	F
1469	HWP1_2_1	R
1470	HYR1_1_1	F
1471	HYR1_1_1	R
1472	INT1a_1_1	F
1473	INT1a_1_1	R
1474	KRE15f_1_1	F
1475	KRE15f_1_1	R
1476	KRE6_1_1	F
1477	KRE6_1_1	R
1478	KRE9_1_1	F
1479	KRE9_1_1	R
1480	MIG1_1_1	F
1481	MIG1_1_1	R
1482	MLS1_1_1	F
1483	MLS1_1_1	R
1484	MP65_1_1	F
1485	MP65_1_1	R
1486	NDE1_1_1	F
1487	NDE1_1_1	R
1488	PFK2_1_1	F
1489	PFK2_1_1	R
1490	PHR1_1_1	F
1491	PHR1_1_1	R
1492	PHR2_1_1	F
1493	PHR2_1_1	R
1494	PHR3_1_1	F
1495	PHR3_1_1	R
1496	PRA1_1_1	F
1497	PRA1_1_1	R
1498	PRS1_1_1	F
1499	PRS1_1_1	R
1500	RBT1_1_1	F
1501	RBT1_1_1	R
1502	RBT4_1_1	F
1503	RBT4_1_1	R
1504	RHO1_1_1	F
1505	RHO1_1_1	R
1506	RNR1_1_1	F
1507	RNR1_1_1	R
1508	RPB7_1_1	F
1509	RPB7_1_1	R
1510	RPL13_1_1	F

SEQ ID NO	Probe name	Direction
1511	RPL13_1_1	R
1512	RVS167_1_1	F
1513	RVS167_1_1	R
1514	SHA3_1_1	F
1515	SHA3_1_1	R
1516	SKN1_1_1	F
1517	SKN1_1_1	R
1518	SRB1_1_1	F
1519	SRB1_1_1	R
1520	TCA1_1_1	F
1521	TCA1_1_1	R
1522	TRP1_1_1	F
1523	TRP1_1_1	R
1524	YAE1_1_1	F
1525	YAE1_1_1	R
1526	YRB1_1_1	F
1527	YRB1_1_1	R
1528	YST1exon2_1_1	F
1529	YST1exon2_1_1	R
1530	CCN1_1_1	F
1531	CCN1_1_1	R
1532	CDC28_1_1	F
1533	CDC28_1_1	R
1534	CLN2_1_1	F
1535	CLN2_1_1	R
1536	CPH1_1_1	F
1537	CPH1_1_1	R
1538	CYB1_1_1	F
1539	CYB1-1_1	R
1540	EFG1_1_1	F
1541	EFG1_1_1	R
1542	MNT1_1_1	F
1543	MNT1_1_1	R
1544	RBF1_1_1	F
1545	RBF1_1_1	R
1546	RBF1_2_1	F
1547	RBF1_2_1	R
1548	RIM101_1_1	F
1549	RIM101_1_1	R
1550	RIM8_1_1	F
1551	RIM8_1_1	R
1552	SEC14_1_1	F
1553	SEC14_1_1	R
1554	SEC4_1_1	F

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SEQ ID NO	Probe name	Direction
1555	SEC4_1_1	R
1556	TUP1_1_1	F
1557	TUP1_1_1	R
1558	YPT1_1_1	F
1559	YPT1_1_1	R
1560	ZNF1CZF1_2_1	F
1561	ZNF1CZF1_2_1	R
1562	arcA_1_1	F
1563	arcA_1_1	R
1564	arcC_1_1	F
1565	arcC_1_1	R
1566		F
1	bkdA_1_1	R
1567	bkdA_1_1	F
1568	cad_1_1	
1569	cad_1_1	R F
1570	camE1_1_1	
1571	camE1_1_1	R
1572	csrA_1_1	F
1573	csrA_1_1	R
1574	dacA_1_1	F
1575	dacA_1_1	R
1576	dfr_1_1	F
1577	dfr_1_1	R
1578	dhoD1a_1_1	F
1579	dhoD1a_1_1	R
1580	ABC-eltA_1_1	F
1581	ABC-eltA_1_1	R
1582	agrBfs_1_1	F
1583	agrBfs_1_1	R
1584	agrCfs_1_1	F
1585	agrCfs_1_1	R
1586	dnaE_1_1	F
1587	dnaE_1_1	R
1588	ebsA_1_1	F
1589	ebsA_1_1	R
1590	ebsB_1_1	F
1591	ebsB_1_1	R
1592	eep_1_1	F
1593	eep_1_1	R
1594	efaR_1_1	F
1595	efaR_1_1	R
1596	gls24_glsB_1_1	F
1597	gls24_glsB_1_1	R
1598	gph_1_1	F

SEQ ID NO	Probe name	Direction
1599	gph_1_1	R
1600	gyrAEf_1_1	F
1601	gyrAEf_1_1	R
1602	metEf_1_1	F
1603	metEf_1_1	R
1604	mntHCb2_1_1	F
1605	mntHCb2_1_1	R
1606	mob2_1_1	F
1607	mob2_1_1	R
1608	mvaD_1_1	F
1609	mvaD_1_1	R
1610	 mvaE_1_1	F
1611	mvaE_1_1	R
1612	parC_1_1	F
1613	parC_1_1	R
1614	pcfG_1_1	F
1615	pcfG_1_1	R
1616	phoZ_1_1	F
1617	phoZ_1_1	R
1618	polC_1_1	F
1619	polC_1_1	R
1620	ptb_1_1	F
1621	ptb_1_1	R
1622	recS1_1_1	F
1623	recS1_1_1	R
1624	rpoN_1_1	F
1625	rpoN_1_1	R
1626	tms_1_1	F
1627	tms_1_1	R
1628	tyrDC_1_1	F
1629	tyrDC_1_1	R
1630	tyrS_1_1	F
1631	tyrS_1_1	R
1632	asa1_1_1	F
1633	asa1_1_1	R
1634	asp1_1_1	F
1635	asp1_1_1	R
1636	cgh_1_1	F
1637	cgh_1_1	R
1638	cylA_1_1	F
1639	cylA_1_1	R
1640	cylB_1_1	F
1641	cylB_1_1	R
1642	cylI_1_1	F

SEQ ID NO	Probe name	Direction
1643	cylI_1_1	R
1644	cylL_cylS_1_1	F
1645	cylL_cylS_1_1	R
1646	cylM_1_1	F
1647	cylM_1_1	R
1648	ace_1_1	F
1649	ace_1_1	R
1650	ef00108_1_1	F
1651	ef00108_1_1	R
1652	ef00109_1_1	F
1653	ef00109_1_1	R
1654	ef0011_1_1	F
1655	ef0011_1_1	R
1656	ef00113_1_1	F
1657	ef00113_1_1	R
1658	ef0012_1_1	F
1659	ef0012_1_1	R
1660	ef0022_1_1	F
1661	ef0022_1_1	R
1662	ef0031_1_1	F
1663	ef0031_1_1	R
1664	ef0032_1_1	F
1665	ef0032_1_1	R
1666	ef0040_1_1	F
1667	ef0040_1_1	R
1668	ef0058_1_1	F
1669	ef0058_1_1	R
1670	enIA_1_1	F
1671	enIA_1_1	R
1672	esa_1_1	F
1673	esa_1_1	R
1674	esp_1_1	F
1675	esp_1_1	R
1676	gelE_1_1	F
1677	gelE_1_1	R
1678	groEL_1_1	F
1679	groEL_1_1	R
1680	groES_1_1	F
1681	groES_1_1	R
1682	rt1_1_1	F
1683	rt1_1_1	R
1684	sala_1_1	F
1685		R
1686	salb_1_1	F

SEQ ID NO	Probe name	Direction
1687	salb_1_1	R
1688	sea1_1_1	F
1689	sea1_1_1	R
1690	sep1_1_1	F
1691	sep1_1_1	R
1692	vicK_1_1	F
1693	vicK_1_1	R
1694	yycH_1_1	F
1695	yycH_1_1	R
1696	yycI_1_1	F
1697	yycI_1_1	R
1698	yycJ_1_1	F
1699	yycJ_1_1	R
1700	bgIB_1_1	F
1701	bgIB_1_1	R
1702	bgIR_1_1	F
1703	bgIR_1_1	R
1704	bgIS_1_1	F
1705	bgIS_1_1	R
1706	efmA_1_1	F
1707	efmA_1_1	R
1708	efmB_1_1	F
1709	efmB_1_1	R
1710	efmC_1_1	F
1711	efmC_1_1	R
1712	mreC_1_1	F
1713	mreC_1_1	R
1714	mreD_1_1	F
1715	mreD_1_1	R
1716	mvaDEfaecium_1_1	F
1717	mvaDEfaecium_1_1	R
1718	mvaEEfaecium_1_1	F
1719	mvaEEfaecium_1_1	R
1720	mvaK1Efaecium_1_1	F
1721	mvaK1Efaecium_1_1	R
1722	mvaK2Efaecium_1_1	F
1723	mvaK2Efaecium_1_1	R
1724	mvaSEfaecium_1_1	F
1725	mvaSEfaecium_1_1	R
1726	orf3_4Efaeciumb_1_1	F
1727	orf3_4Efaeciumb_1_1	R
1728	orf6_7Efaecium_1_1	F
1729	orf6_7Efaecium_1_1	R
1730	orf7_8Efaecium_1_1	F

SEQ ID NO	Probe name	Direction
1731	orf7_8Efaecium_1_1	R
1732	orf9_10Efaecium_1_1	F
1733	orf9_10Efaecium_1_1	R
1734	entA_entI_1_1	F
1735	entA_entI_1_1	R
1736	entD_1_1	F
1737	entD_1_1	R
1738	entR_1_1	F
1739	entR_1_1	R
1740	oep_1_1	F
1741	oep_1_1	R
1742	sagA_1_2	F
1743	sagA_1_2	R
1744	atsA_1_1	F
1745	atsA_1_1	R
1746	atsB_1_1	F
1747	atsB_1_1	R
1748	budC_1_1	F
1749	budC_1_1	R
1750	citA_1_1	F
1751	citA_1_1	R
1752	citW_1_1	F
1753	citW_1_1	R
1754	citX_1_1	F
1755	citX_1_1	R
1756	dalD_1_1	F
1757	dalD_1_1	R
1758	dalK_1_1	F
1759	dalK_1_1	R
1760	dalT_1_1	F
1761	dalT_1_1	R
1762	acoA_1_1	F
1763	acoA_1_1	R
1764	acoB_1_1	F
1765	acoB_1_1	R
1766	acoC_1_1	F
1767	acoC_1_1	R
1768	ahlK_1_1	F
1769	ahlK_1_1	R
1770	fimK_1_1	F
1771	 fimK_1_1	R
1772	glfKPN2_1_1	F
1773	glfKPN2_1_1	R
1774		F

SEQ ID NO	Probe name	Direction
1775	ltrA_1_1	R
1776	mdcC_1_1	F
1777	mdcC_1_1	R
1778	mdcF_1_1	F
1779	mdcF_1_1	R
1780	mdcH_1_1	F
1781	mdcH_1_1	R
1782	mrkA_1_1	F
1783	mrkA_1_1	R
1784	mtrK_1_1	F
1785	mtrK_1_1	R
1786	nifF_1_1	F
1787	nifF_1_1	R
1788	nifK_1_1	F
1789	nifK_1_1	R
1790	nifN_1_1	F
1791	nifN_1_1	R
1792	tyrP_1_1	F
1793	tyrP_1_1	R
1794	ureA_1_1	F
1795	ureA_1_1	R
1796	wbbO_1_1	F
1797	wbbO_1_1	R
1798	wza_1_1	F
1799	wza_1_1	R
1800	wzb_1_1	F
1801	wzb_1_1	R
1802	wzmKPN2_1_1	F
1803	wzmKPN2_1_1	R
1804	wztKPN2_1_1	F
1805	wztKPN2_1_1	R
1806	yojH_1_1	F
1807	yojH_1_1	R
1808	liac_1_1	F
1809	liac_1_1	R
1810	cim_1_1	F
1811	cim_1_1	R
1812	aldA_1_1	F
1813	aldA_1_1	R
1814	aldA_2_1	F
1815	aldA_2_1	R
1816	hemly_1_1	F
1817	hemly_1_1	R
1818	pSL017_1_1	F

SEQ ID NO	Probe name	Direction
1819	pSL017_1_1	R
1820	pSL020_1_1	F
1821	pSL020_1_1	R
1822	rcsA_1_1	F
1823	rcsA_1_1	R
1824	rmlC_1_1	F
1825	rmIC_1_1	R
1826	rmID_1_1	F
1827	rmID_1_1	R
1828	waaG_1_1	F
1829	waaG_1_1	R
1830	wbbD_1_1	F
1831	wbbD_1_1	R
1832	wbbM_1_1	F
1833	wbbM_1_1	R
1834	wbbN_1_1	F
1835	wbbN_1_1	R
1836	wbdA_1_1	F
1837	wbdA_1_1	R
1838	wbdC_1_1	F
1839	wbdC_1_1	R
1840	wztKpn_1_1	F
1841	wztKpn_1_1	R
1842	yibD_1_1	F
1843	yibD_1_1	R
1844	cymA_1_1	F
1845	cymA_1_1	R
1846	cymD_1_1	F
1847	cymD_1_1	R
1848	cymE_1_1	F
1849	cymE_1_1	R
1850	cymH_1_1	F
1851	cymH_1_1	R
1852	cymI_1_1	F
1853	cymI_1_1	R
1854	cymJ_1_1	F
1855	cymJ_1_1	R
1856	ddrA_1_1	F
1857	ddrA_1_1	R
1858	fdt-1_1_1	F
1859	fdt-1_1_1	R
1860	fdt-2_1_1	F
1861	fdt-2_1_1	R
1862	fdt-3_1_1	F

SEQ ID NO	Probe name	Direction
1863	fdt-3_1_1	R
1864	gatY_1_1	F
1865	gatY_1_1	R
1866	hydH_1_1	F
1867	hydH_1_1	R
1868	masA_1_1	F
1869	masA_1_1	R
1870	nasA_1_1	F
1871	nasA_1_1	R
1872	nasE_1_1	F
1873	nasE_1_1	R
1874	nasF_1_1	F
1875	nasF_1_1	R
1876	pehX_1_1	F
1877	pehX_1_1	R
1878	pelX_1_1	F
1879	pelX_1_1	R
1880	tagH_1_1	F
1881	tagH_1_1	R
1882	tagK_1_1	F
1883	tagK_1_1	R
1884	tagT_1_1	F
1885	tagT_1_1	R
1886	glpR_1_1	F
1887	glpR_1_1	R
1888	lasRb_1_1	F
1889	lasRb_1_1	R
1890	OrfX_1_1	F
1891	OrfX_1_1	R
1892	pa0260_1_1	F
1893	pa0260_1_1	R
1894	pa0572_1_1	F
1895	pa0572_1_1	R
1896	pa0625_1_1	F
1897	pa0625_1_1	R
1898	pa0636_1_1	F
1899	pa0636_1_1	R
1900	pa1046_1_1	F
1901	pa1046_1_1	R
1902	pa1069_1_1	F
1903	pa1069_1_1	R
1904	pa1846_1_1	F
1905	pa1846_1_1	R
1906	pa3866_1_1	F

SEQ ID NO	Probe name	Direction
1907	pa3866_1_1	R
1908	pa4082_1_1	F
1909	pa4082_1_1	R
1910	pilAp_1_1	F
1911	pilAp_1_1	R
1912	PilAp2_1_1	F
1913	PilAp2_1_1	R
1914	pilC_1_1	F
1915	pilC_1_1	R
1916	PstP_1_1	F
1917	PstP_1_1	R
1918	purK_1_1	F
1919	purK_1_1	R
1920	uvrDII_1_1	F
1921	uvrDII_1_1	R
1922	vsmI_1_1	F
1923	vsmI_1_1	R
1924	vsmR_1_2	F
1925	vsmR_1_2	R
1926	xcpX_1_1	F
1927	xcpX_1_1	R
1928	aprA_1_1	F
1929	aprA_1_1	R
1930	aprE_1_1	F
1931	aprE_1_1	R
1932	ctx_1_2	F
1933	ctx_1_2	R
1934	algB_1_1	F
1935	algB_1_1	R
1936	algN_1_1	F
1937	algN_1_1	R
1938	algR_1_1	F
1939	algR_1_1	R
1940	ExoS_1_1	F
1941	ExoS_1_1	R
1942	fpvA_1_1	F
1943	fpvA_1_1	R
1944	lasRa_1_1	F
1945	lasRa_1_1	R
1946	lipA_1_1	F
1947	lipA_1_1	R
1948	lipH_1_1	F
1949	lipH_1_1	R
1950	Orf159_1_2	F

SEQ ID NO	Probe name	Direction
1951	Orf159_1_2	R
1952	Orf252_1_1	F
1953	Orf252_1_1	R
1954	pchG_1_1	F
1955	pchG_1_1	R
1956	PhzA_1_1	F
1957	PhzA_1_1	R
1958	PhzB_1_1	F
1959	PhzB_1_1	R
1960	PLC_1_1	F
1961	PLC_1_1	R
1962	plcN_1_1	F
1963	plcN_1_1	R
1964	plcR_1_1	F
1965	plcR_1_1	R
1966	pvdD_1_1	F
1967	pvdD_1_1	R
1968	pvdF_1_2	F
1969	pvdF_1_2	R
1970	pyocinS1_1_1	F
1971	pyocinS1_1_1	R
1972	pyocinS1im_1_1	F
1973	pyocinS1im_1_1	R
1974	pyocinS2_1_1	F
1975	pyocinS2_1_1	R
1976	pys2_1_1	F
1977	pys2_1_1	R
1978	pys2_2_1	F
1979	pys2_2_1	R
1980	rbf303_1_1	F
1981	rbf303_1_1	R
1982	rhIA_1_1	F
1983	rhIA_1_1	R
1984	rhlB_1_1	F
1985	rhlB_1_1	R
1986	rhIR_1_1	F
1987	rhlR_1_1	R
1988	TnAP41_1_2	F
1989	TnAP41_1_2	R
1990	toxA_1_1	F
1991	toxA_1_1	R
1992	cap1EStrpneu_1_1	F
1993	cap1EStrpneu_1_1	R
1994	cap1FStrpneu_1_1	F

SEQ ID NO	Probe name	Direction
1995	cap1FStrpneu_1_1	R
1996	cap1GStrpneu_1_1	F
1997	cap1GStrpneu_1_1	R
1998	cap3AStrpneu_1_1	F
1999	cap3AStrpneu_1_1	R
2000	cap3BStrpneu_1_1	F
2001	cap3BStrpneu_1_1	R
2002	celAStrpneu_1_1	F
2003	celAStrpneu_1_1	R
2004	celBStrpneu_1_1	F
2005	celBStrpneu_1_1	R
2006	cglAStrpneu_1_1	F
2007	cglAStrpneu_1_1	R
2008	cglBStrpneu_1_1	F
2009	cglBStrpneu_1_1	R
2010	cglCStrpneu_1_1	F
2011	cglCStrpneu_1_1	R
2012	cglDStrpneu_1_1	F
2013	cglDStrpneu_1_1	R
2014	cinA_1_1	F
2015	cinA_1_1	R
2016	cps14EStrpneum_1_1	F
2017	cps14EStrpneum_1_1	R
2018	cps14FStrpneum_1_1	F
2019	cps14FStrpneum_1_1	R
2020	cps14GStrpneum_1_1	F
2021	cps14GStrpneum_1_1	R
2022	cps14HStrpneum_1_1	F
2023	cps14HStrpneum_1_1	R
2024	cps19aHStrpneum_1_1	F
2025	cps19aHStrpneum_1_1	R
2026	cps19aIStrpneum_1_1	F
2027	cps19aIStrpneum_1_1	R
2028	cps19aKStrpneum_1_1	F
2029	cps19aKStrpneum_1_1	R
2030	cps19fGStrpneum_1_1	F
2031	cps19fGStrpneum_1_1	R
2032	cps23fGStrpneum_1_1	F
2033	cps23fGStrpneum_1_1	R
2034	dexB_1_1	F
2035	dexB_1_1	R
2036	dinF_1_1	F
2037	dinF_1_1	R
2038	1760Strpneu_1_1	F

SEQ ID NO	Probe name	Direction
2039	1760Strpneu_1_1	R
2040	acyPStrpneu_1_1	F
2041	acyPStrpneu_1_1	R
2042	endAStrpneu_1_1	F
2043	endAStrpneu_1_1	R
2044	exoAStrpneu_1_1	F
2045	exoAStrpneu_1_1	R
2046	exp72_1_1	F
2047	exp72_1_1	R
2048	fnlAStrpneu_1_1	F
2049	fnlAStrpneu_1_1	R
2050	fnlBStrpneu_1_1	F
2051	fnlBStrpneu_1_1	R
2052	fnlCStrpneu_1_1	F
2053	fnlCStrpneu_1_1	R
2054	gct18Strpneum_1_1	F
2055	gct18Strpneum_1_1	R
2056	hexB1_1_1	F
2057	hexB1_1_1	R
2058	hftsHstrpneu_1_1	F
2059	hftsHstrpneu_1_1	R
2060	immunofrag1Strpneu_1_1	F
2061	immunofrag1Strpneu_1_1	R
2062	immunofrag2Strpneu_2_1	F
2063	immunofrag2Strpneu_2_1	R
2064	immunofrag3Strpneu_2_1	F
2065	immunofrag3Strpneu_2_1	R
2066	kdtBStrpneu_1_1	F
2067	kdtBStrpneu_1_1	R
2068	lysAStrpneu_1_1	F
2069	lysAStrpneu_1_1	R
2070	pcpBStrpneu_1_1	F
2071	pcpBStrpneu_1_1	R
2072	pflCStrpneu_1_1	F
2073	pflCStrpneu_1_1	R
2074	plpA_1_1	F
2075	plpA_1_1	R
2076	prtA1Strpneu_1_1	F
2077	prtA1Strpneu_1_1	R
2078	pspC1Strpneu_1_1	F
2079	pspC1Strpneu_1_1	R
2080	pspC2_1_1	F
2081	pspC2_1_1	R
2082	purRStrpneu_1_1	F

SEQ ID NO	Probe name	Direction
2083	purRStrpneu_1_1	R
2084	pyrDAStrpneum_1_1	F
2085	pyrDAStrpneum_1_1	R
2086	SP0828Strpneu_1_1	F
2087	SP0828Strpneu_1_1	R
2088	SP0830Strpneu_1_1	F
2089	SP0830Strpneu_1_1	R
2090	SP0833Strpneu_1_1	F
2091	SP0833Strpneu_1_1	R
2092	SP0837_38Strpneu_1_1	F
2093	SP0837_38Strpneu_1_1	R
2094	SP0839Strpneu_1_1	F
2095	SP0839Strpneu_1_1	R
2096	ugdStrpneu_1_1	F
2097	ugdStrpneu_1_1	R
2098	uncC_1_1	F
2099	uncC_1_1	R
2100	vicXStrepneu_1_1	F
2101	vicXStrepneu_1_1	R
2102	wchA6bStrpneum_1_1	F
2103	wchA6bStrpneum_1_1	R
2104	wci4Strpneum_1_1	F
2105	wci4Strpneum_1_1	R
2106	wciK4Strpneum_1_1	F
2107	wciK4Strpneum_1_1	R
2108	wciL4Strpneum_1_1	F
2109	wciL4Strpneum_1_1	R
2110	wciN6bStrpneum_1_1	F
2111	wciN6bStrpneum_1_1	R
2112	wciO6bStrpneum_1_1	F
2113	wciO6bStrpneum_1_1	R
2114	wciP6bStrpneum_1_1	F
2115	wciP6bStrpneum_1_1	R
2116	wciY18Strpneum_1_1	F
2117	wciY18Strpneum_1_1	R
2118	wzdbStrpneum_1_1	F
2119	wzdbStrpneum_1_1	R
2120	wze6bStrpneum_1_1	F
2121	wze6bStrpneum_1_1	R
2122	wzy18Strpneum_1_1	F
2123	wzy18Strpneum_1_1	R
2124	wzy4Strpneum_1_1	F
2125	wzy4Strpneum_1_1	R
2126	wzy6bStrpneum_1_1	F

SEQ ID NO	Probe name	Direction
2127	wzy6bStrpneum_1_1	R
2128	xpt_1_1	F
2129	xpt_1_1	R
2130	igaStrpneu_1_1	F
2131	igaStrpneu_1_1	R
2132	lytA_1_1	F
2133	lytA_1_1	R
2134	nanA_1_1	F
2135	nanA_1_1	R
2136	nanBStrpneu_1_1	F
2137	nanBStrpneu_1_1	R
2138	pcpCStrpneu_1_1	F
2139	pcpCStrpneu_1_1	R
2140	ply_1_1	F
2141	ply_1_1	R
2142	prtAStrpneu_1_1	F
2143	prtAStrpneu_1_1	R
2144	pspA_1_2	F
2145	pspA_1_2	R
2146	SP0834Strpneu_1_1	F
2147	SP0834Strpneu_1_1	R
2148	SP0834Strpneu_1_2	F
2149	SP0834Strpneu_1_2	R
2150	sphtraStrpneu_1_1	F
2151	sphtraStrpneu_1_1	R
2152	wciJStrpneu_1_1	F
2153	wciJStrpneu_1_1	R
2154	wziyStrpneu_1_1	F
2155	wziyStrpneu_1_1	R
2156	wzxStrpneu_1_1	F
2157	wzxStrpneu_1_1	R
2158	cpsA1Strgal_1_1	F
2159	cpsA1Strgal_1_1	R
2160	cpsB1Strgal_1_1	F
2161	cpsB1Strgal_1_1	R
2162	cpsC1Strgal_1_1	F
2163	cpsC1Strgal_1_1	R
2164	cpsD1Strgal_1_1	F
2165	cpsD1Strgal_1_1	R
2166	cpsE1Strgal_1_1	F
2167	cpsE1Strgal_1_1	R
2168	cpsG1Strgal_1_1	F
2169	cpsG1Strgal_1_1	R
2170	cpsIStragal_1_1	F

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SEQ ID NO	Probe name	Direction
2171	cpsIStragal_1_1	R
2172	cpsJStragal_1_1	F
2173	cpsJStragal_1_1	R
2174	cpsKStragal_1_1	F
2175	cpsKStragal_1_1	R
2176	cpsMStragal_1_1	F
2177	cpsMStragal_1_1	R
2178	cpsYStragal_1_1	F
2179	cpsYStragal_1_1	R
2180	cpsYStragal_2_1	F
2181	cpsYStragal_2_1	R
2182	cylBStraga_1_1	F
2183	cylBStraga_1_1	R
2184	cylEStraga_1_1	F
2185	cylEStraga_1_1	R
2186	cylFStraga_1_1	F
2187	cylFStraga_1_1	R
2188	cylHStraga_1_1	F
2189	cylHStraga_1_1	R
2190	cylIStraga_1_1	F
2191	cylIStraga_1_1	R
2192	cylJStraga_1_1	F
2193	cylJStraga_1_1	R
2194	cylKStraga_1_1	F
2195	cylKStraga_1_1	R
2196	0487Straga_1_1	F
2197	0487Straga_1_1	R
2198	0488Straga_1_1	F
2199	0488Straga_1_1	R
2200	0493Straga_1_1	F
2201	0493Straga_1_1	R
2202	0495Straga_1_1	F
2203	0495Straga_1_1	R
2204	0498Straga_1_1	F
2205	0498Straga_1_1	R
2206	0500Straga_1_1	F
2207	0500Straga_1_1	R
2208	0502Straga_1_1	F
2209	0502Straga_1_1	R
2210	0504Straga_1_1	F
2211	0504Straga_1_1	R
2212	folDStraga_1_1	F
2212	folDStraga_1_1	R
2214	neuA1Strgal_1_1	F
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SEQ ID NO	Probe name	Direction
2215	neuA1Strgal_1_1	R
2216	neuB1Strgal_1_1	F
2217	neuB1Strgal_1_1	R
2218	neuC1Strgal_1_1	F
2219	neuC1Strgal_1_1	R
2220	neuD1Strgal_1_1	F
2221	neuD1Strgal_1_1	R
2222	recNStraga_1_1	F
2223	recNStraga_1_1	R
2224	ileSStraga_1_1	F
2225	ileSStraga_1_1	R
2226	CAMPfactor_1_1	F
2227	CAMPfactor_1_1	R
2228	CAMPfactor_2_1	F
2229	CAMPfactor_2_1	R
2230	0499Straga_1_1	F
2231	0499Straga_1_1	R
2232	hylStragal_1_1	F
2233	hylStragal_1_1	R
2234	lipStragal_1_1	F
2235	lipStragal_1_1	R
2236	cyclStrpyog_1_1	F
2237	cyclStrpyog_1_1	R
2238	fah_rph_hlo_Strpyog_1_1	F
2239	fah_rph_hlo_Strpyog_1_1	R
2240	int_1_1	F
2241	int_1_1	R
2242	int315.5_1_1	F
2243	int315.5_1_1	  R
2244	murEStrpyog_1_1	F
2245	murEStrpyog_1_1	R
2246	oppA_1_1	F
2247	oppA_1_1	R
2248	oppCStrpyog_1_1	F
2249	oppCStrpyog_1_1	R
2250	oppD_1_1	F
2251	oppD_1_1	R
2252	SPy0382Strpyog_1_1	F
2253	SPy0382Strpyog_1_1	R
2254	SPy0390Strpyog_1_1	F
2255	SPy0390Strpyog_1_1	
2256		R F
2257	SpyM3_1351_1_1 SpyM3_1351_1_1	
Į.	SpyM3_1351_1_1 vioVStravog_1_1	R
2258	vicXStrpyog_1_1	F

SEQ ID NO	Probe name	Direction
2259	vicXStrpyog_1_1	R
2260	DNaseIStrpyog_1_1	F
2261	DNaseIStrpyog_1_1	R
2262	fba2Strpyog_1_1	F
2263	fba2Strpyog_1_1	R
2264	fhuAStrpyog_1_1	F
2265	fhuAStrpyog_1_1	R
2266	fhuB1Strpyog_1_1	F
2267	fhuB1Strpyog_1_1	R
2268		F
1	fhuDStrpyog_1_1	
2269	fhuDStrpyog_1_1	R F
2270	fhuGStrpyog_1_1	
2271	fhuGStrpyog_1_1	R
2272	hylA_1_1	F
2273	hylA_1_1	R
2274	hylP_1_1	F
2275	hylP_1_1	R
2276	hylp2_1_1	F
2277	hylp2_1_1	R
2278	oppB_1_1	F
2279	oppB_1_1	R
2280	ropB_1_1	F
2281	ropB_1_1	R
2282	scpAStrpyog_1_1	F
2283	scpAStrpyog_1_1	R
2284	sloStrpyog_1_1	F
2285	sloStrpyog_1_1	R
2286	smez-4Strpyog_1_1	F
2287	smez-4Strpyog_1_1	R
2288	sof_1_1	F
2289	sof_1_1	R
2290	sof_2_1	F
2291	sof_2_1	R
2292	speA_1_1	F
2293	speA_1_1	R
2294	speB2Strpyog_1_1	F
2295	speB2Strpyog_1_1	R
2296	speCStrpyog_1_1	F
2297	speCStrpyog_1_1	R
2298	speJStrpyog_1_1	F
2299	speJStrpyog_1_1	R
2300	srtBStrpyog_1_1	F
2301	srtBStrpyog_1_1	r R
2302	srtCStrpyog_1_1	F
2302	SICSUPY09_1_1	<u>l'</u>

SEQ ID NO	Probe name	Direction
2303	srtCStrpyog_1_1	R
2304	srtEStrpyog_1_1	F
2305	srtEStrpyog_1_1 srtEStrpyog_1_1	r R
2306	srtFStrpyog_1_1	F
2307	srtFStrpyog_1_1	r R
2308	srtGStrpyog_1_1	F
2309	srtGStrpyog_1_1 srtGStrpyog_1_1	R
2310		F
2311	srtIStrpyog_1_1 srtIStrpyog_1_1	R
2312	., - =	F
2312	srtKStrpyog_1_1	F  R
2314	srtKStrpyog_1_1	F
1	srtRStrpyog_1_1	R
2315	srtRStrpyog_1_1	F
2316	srtTStrpyog_1_1	l
2317	srtTStrpyog_1_1	R F
2318	vicKStrpyog_1_1	I .
2319	vicKStrpyog_1_1	R
2320	573Stprmut_1_1	F
2321	573Stprmut_1_1	R
2322	580SStprmut_1_1	F
2323	580SStprmut_1_1	R
2324	581_582SStprmut_1_1	F
2325	581_582SStprmut_1_1	R -
2326	584SStprmut_1_1	F
2327	584SStprmut_1_1	R
2328	dltAStrmut_1_1	F
2329	dltAStrmut_1_1	R
2330	dltBStrmut_1_1	F
2331	dltBStrmut_1_1	R
2332	dltCppx1Strmut_1_1	F
2333	dltCppx1Strmut_1_1	R
2334	dltDStrmut_1_1	F
2335	dltDStrmut_1_1	R
2336	lichStrbov_1_1	F
2337	lichStrbov_1_1	R
2338	lytRStprmut_1_1	F
2339	lytRStprmut_1_1	R
2340	lytSStprmut_1_1	F
2341	lytSStprmut_1_1	R
2342	pepQStrrmut_1_1	F
2343	pepQStrrmut_1_1	R
2344	pflCStrmut_1_1	F
2345	pflCStrmut_1_1	R
2346	recNStprmut_1_1	F

SEQ ID NO	Probe name	Direction
2347	recNStprmut_1_1	R
2348	ytqBStrmut_1_1	F
2349	ytqBStrmut_1_1	R
2350	hlyXStrmut_1_1	F
2351	hlyXStrmut_1_1	R
2352	igaStrmitis_1_1	F
2353	igaStrmitis_1_1	R
2354	igaStrsanguis_1_1	F
2355	igaStrsanguis_1_1	R
2356	perMStrmut_1_1	F
2357	perMStrmut_1_1	R
2358	atfA_1_1	F
2359	atfA_1_1	R
2360	atfB_1_1	F
2361	atfB_1_1	R
2362	atfC_1_1	F
2363	atfC_1_1	R
2364	ccmPrmi1_1_1	F
2365	ccmPrmi1_1_1	R
2366	cyaPrmi_1_1	F
2367	cyaPrmi_1_1	R
2368	aad_1_1	F
2369	aad_1_1	R
2370	flfB_1_1	F
2371	flfB_1_1	R
2372	flfD_1_1	F
2373	flfD_1_1	R
2374	flfN_1_1	F
2375	flfN_1_1	R
2376	flhD_1_1	F
2377	flhD_1_1	R
2378	floA_1_1	F
2379	floA_1_1	R
2380	ftsK_1_1	F
2381	ftsK_1_1	R
2382	gstB_1_1	F
2383	gstB_1_1	R
2384	hemCPrmi_1_1	F
2385	hemCPrmi_1_1	R
2386	hemDPrmi_1_1	F
2387	hemDPrmi_1_1	R
2388	hev_1_1	F
2389	hev_1_1	R
2390	katA_1_1	F

SEQ ID NO	Probe name	Direction
2391	katA_1_1	R
2392	lpp1_1_1	ļ <sub>F</sub>
2393	pp1_1_1	R
2394	menE_1_1	F
2395	menE_1_1	R
2396	mfd_1_1	F
2397	mfd_1_1	R
2398	nrpA_1_1	F
2399	nrpA_1_1	R
2400	nrpB_1_1	F
2401	nrpB_1_1	R
2402	nrpG_1_1	F
2403	nrpG_1_1	R
2404	nrpS_1_1	F
2405	nrpS_1_1	R
2406	nrpT_1_1	F
2407	nrpT_1_1	R
2408	nrpU_1_1	F
2409	nrpU_1_1	R
2410	pat_1_1	F
2411	pat_1_1	R
2412	pmfA_1_1	F
2413	pmfA_1_1	R
2414	pmfC_1_1	F
2415	pmfC_1_1	R
2416	pmfE_1_1	F
2417	pmfE_1_1	R
2418	ppaA_1_1	F
2419	ppaA_1_1	R
2420	rsbA_1_1	F
2421	rsbA_1_1	R
2422	rsbC_1_1	F
2423	rsbC_1_1	R
2424	speB_1_1	F
2425	speB_1_1	R
2426	stmA_1_1	F
2427	stmA_1_1	R
2428	stmB_1_1	F
2429	stmB_1_1	R
2430	terA_1_1	F
2431	terA_1_1	R
2432	terD_1_1	F
2433	terD_1_1	R
2434	umoA_1_1	F

SEQ ID NO	Probe name	Direction
2435	umoA_1_1	R
2436	umoB_1_1	F
2437	umoB_1_1	R
2438	umoC_1_1	ļF
2439	umoC_1_1	R
2440	ureR_1_1	F
2441	ureR_1_1	R
2442	xerC_1_1	F
2443	xerC_1_1	R
2444	ygbA_1_1	F
2445	ygbA_1_1	R
2446	flaA_1_1	F
2447	flaA_1_1	R
2448	flaD_1_1	F
2449	flaD_1_1	R
2450	fliA_1_1	F
2451	fliA_1_1	R
2452	hpmA_1_1	F
2453	hpmA_1_1	R
2454	hpmB_1_1	F
2455	hpmB_1_1	R
2456	lpsPrmi_1_1	F
2457	lpsPrmi_1_1	R
2458	mrpA_1_1	F
2459	mrpA_1_1	R
2460	mrpB_1_1	F
2461	mrpB_1_1	R
2462	mrpC_1_1	F
2463	mrpC_1_1	R
2464	mrpD_1_1	F
2465	mrpD_1_1	R
2466	mrpE_1_1	F
2467	mrpE_1_1	R
2468	mrpF_1_1	F
2469	mrpF_1_1	R
2470	mrpG_1_1	F
2471	mrpG_1_1	R
2472	mrpH_1_1	F
2473	mrpH_1_1	R
2474	mrpI_1_1	F
2475	mrpI_1_1	R
2476	mrpJ_1_1	F
2477	mrpJ_1_1	R
2478	patA_1_1	F

SEQ ID NO	Probe name	Direction
2479	patA_1_1	R
2480	putA_1_1	F
2481	putA_1_1	R
2482	uca_1_1	F
2483	uca_1_1	R
2484	ureDPrmi_1_1	F
2485	ureDPrmi_1_1	R
2486	ureEPrmi_1_1	F
2487	ureEPrmi_1_1	R
2488	ureFPrmi_1_1	F
2489	ureFPrmi_1_1	R
2490	zapA_1_1	F
2491	zapA_1_1	R
2492	zapB_1_1	F
2493	zapB_1_1	R
2494	zapD_1_1	F
2495	zapD_1_1	R
2496	zapE_1_1	F
2497	zapE_1_1	R
2498	envZPrvu_1_1	F
2499	envZPrvu_1_1	R
2500	frdC_1_1	F
2501	frdC_1_1	R
2502	frdD_1_1	F
2503	frdD_1_1	R
2504	infBPrvu_1_1	F
2505	infBPrvu_1_1	R
2506	lad_1_1	F
2507	lad_1_1	R
2508	tna2_1_1	F
2509	tna2_1_1	R
2510	end_1_1	F
2511	end_1_1	R
2512	pqrA_1_1	F
2513	pqrA_1_1	R
2514	urg_1_1	F
2515	urg_1_1	R
2516	blaIMP-7_1_1	F
2517	blaIMP-7_1_1	R
2518	mecISepid_1_1	F
2519	mecISepid_1_1	R
2520	blaOXA-10_1_2	F
2521	blaOXA-10_1_2	R
2522	blaB_1_1	F

SEQ ID NO	Probe name	Direction
2523	blaB_1_1	R
2524	ampC_1_1	F
2525	ampC_1_1	R
2526	I-blaR_1_1	F
2527	I-blaR_1_1	R
2528	blaOXA-32_1_1	F
2529	blaOXA-32_1_1	R
2530	bla-CTX-M-22_1_1	F
2531	bla-CTX-M-22_1_1	R
2532	pbp2aStrpneu_1_1	F
2533	pbp2aStrpneu_1_1	R
2534	blaSHV-1_1_1	F
2535	blaSHV-1_1_1	R
2536	blaOXA-2_1_1	F
2537	blaOXA-2_1_1	R
2538	blaRShaemolyt_1_1	F
2539	blaRShaemolyt_1_1	R
2540	blaIMP-7_1_2	F
2541	blaIMP-7_1_2	R
2542	I-mecR_1_1	F
2543	I-mecR_1_1	R
2544	blaOXY_1_1	F
2545	blaOXY_1_1	R
2546	dacCStrpyog_1_1	F
2547	dacCStrpyog_1_1	R
2548	femA_1_1	F
2549	femA_1_1	R
2550	mecA_1_1	F
2551	mecA_1_1	R
2552	blaIShaemolyt_1_1	F
2553	blaIShaemolyt_1_1	R
2554	blavim_1_1	F
2555	blavim_1_1	R
2556	pbp2b_1_1	F
2557	pbp2b_1_1	R
2558	pbp2primeSepid_1_1	F
2559	pbp2primeSepid_1_1	R
2560	pbp2x_1_1	F
2561	pbp2x_1_1	R
2562	pbp3Saureuc_1_1	F
2563	pbp3Saureuc_1_1	R
2564	pbp4_1_1	F
2565	pbp4_1_1	R
2566	pbp5Efaecium_1_1	F

SEQ ID NO	Probe name	Direction
2567	pbp5Efaecium_1_1	R
2568	pbpC_1_1	F
2569	pbpC_1_1	R
2570	I-mecI_1_1	F
2571	I-mecI_1_1	R
2572	pbp1a_1_1	F
2573	pbp1a_1_1	R
2574	I-blaI_1_1	F
2575	I-blaI_1_1	R
2576	blaTEM-106_1_1	F
2577	blaTEM-106_1_1	R
2578	blaOXY-KLOX_1_1	F
2579	blaOXY-KLOX_1_1	R
2580	ftsWEF_1_1	F
2581	ftsWEF_1_1	R
2582	fmhB_1_1	F
2583	fmhB_1_1	R
2584	cumA_1_1	F
2585	cumA_1_1	R
2586	femBShaemolyt_1_1	F
2587	femBShaemolyt_1_1	R
2588	blaPER-1_1_1	F
2589	blaPER-1_1_1	R
2590	bla_FOX-3_1_1	F
2591	bla_FOX-3_1_1	R
2592	blaA_1_1	F
2593	blaA_1_1	R
2594	psrb_1_1	F
2595	psrb_1_1	R
2596	fmhA_1_1	F
2597	fmhA_1_1	R
2598	mecR1Sepid_1_1	F
2599	mecR1Sepid_1_1	R
2600	blaZ_1_1	F
2601	blaZ_1_1	R
2602	blaOXA-1 1 1	F
2603	blaOXA-1_1_1	R
2604	fox-6_1_1	F
2605	fox-6_1_1	R
2606	blaPrmi_1_1	F
2607	blaPrmi_1_1	R
2608	aacA_aphDStwar_1_1	F
2609	aacA_aphDStwar_1_1	R
2610	aacC1_1_2	F

SEQ ID NO	Probe name	Direction
2611	aacC1_1_2	R
2612	aacC2_1_1	F
2613	aacC2_1_1	R
2614	strB_1_1	F
2615	strB_1_1	R
2616	aadA_1_1	F
2617	aadA_1_1	R
2618	aadB_1_2	F
2619	aadB_1_2	R
2620	aadD_1_1	F
2621	aadD_1_1	R
2622	aacA4_1_2	F
2623	aacA4_1_2	R
2624	strA_1_1	F .
2625	strA_1_1	R
2626	aph-A3_1_1	F
2627	aph-A3_1_1	R
2628	aacC1_1_1	F
2629	aacC1_1_1	R
2630	aacA4_1_1	F
2631	aacA4_1_1	R
2632	aacA-aphD_1_1	F
2633	aacA-aphD_1_1	R
2634	I-spc_1_1	F
2635	I-spc_1_1	R
2636	aphA3_1_1	F
2637	aphA3_1_1	R
2638	ermC_1_1	F
2639	ermC_1_1	R
2640	linB_1_1	F
2641	linB_1_1	R
2642	satSA_1_1	F
2643	satSA_1_1	R
2644	mdrSA_1_1	F
2645	mdrSA_1_1	R
2646	I-linA_1_1	F
2647	I-linA_1_1	R
2648	ermB_1_2	F
2649	ermB_1_2	R
2650	ermA_1_1	F
2651	ermA_1_1	R
2652	satA_1_1	F
2653	satA_1_1	R
2654	msrA_1_1	F

SEQ ID NO	Probe name	Direction
2655	msrA_1_1	R
2656	mphBM_1_1	F
2657	mphBM_1_1	R
2658	mefA_1_1	F
2659	mefA_1_1	R
2660	mrx_1_1	F
2661	mrx_1_1	R
2662	dfrStrpneu_1_1	F
2663	dfrStrpneu_1_1	R
2664	dfrA_1_1	F
2665		R
2666	cmlA5_1_1	F
2667		R
2668		F
2669		R
2670	cat_1_1	F
2671		R
2672		F
2673	tetAJ_1_1	R
2674	tetL_1_1	F
2675		R
2676		F
2677		R
2678	vanH(tn)_1_1	F
2679		R
2680	vanA_1_1	F
2681	vanA_1_1	R
2682	vanHB2_1_1	F
2683-	vanHB2_1_1	R
2684		F
2685		R
2686	vanRB2_1_1	F
2687	vanRB2_1_1	R
2688	vanS(tn)_1_1	F
2689	vanS(tn)_1_1	R
2690	vanSB2_1_1	F
2691	vanSB2_1_1	R
2692		F
2693		R
2694		F
2695		R
2696		F
2697		R
2698	vanXB2_1_1	F

SEQ ID NO	Probe name	Direction
2699	vanXB2_1_1	R
2700	vanY(tn)_1_1	F
2701	vanY(tn)_1_1	R
2702	vanYB2_1_1	F
2703	vanYB2_1_1	R
2704	vanB_1_1	F
2705	vanB_1_1	R
2706	vanZ(tn)_1_1	F
2707	vanZ(tn)_1_1	R
2708	vanC-2_1_1	F
2709	vanC-2_1_1	R
2710	vanX(tn)_1_1	F
2711		R
2712		F
2713		R
2714	mexB_1_2	F
2715	mexB_1_2	R
2716	I-qacA_1_1	F
2717	I-qacA_1_1	R
2718	sulI_1_1	F
2719	sulI_1_1	R
2720	sul_1_1	F
2721	sul_1_1	R
2722	cadBStalugd_1_1	F
2723	cadBStalugd_1_1	R
2724	mexA_1_1	F
2725	mexA_1_1	R
2726	acrR_1_1	F
2727	acrR_1_1	R
2728	emeA_1_1	F
2729	emeA_1_1	R
2730	acrA_1_1	F
2731	acrA_1_1	R
2732	rtn_1_1	F
2733	rtn_1_1	R
2734	abcXStrpmut_1_1	F
2735	abcXStrpmut_1_1	R
2736	qacEdelta1_1_1	F
2737	qacEdelta1_1_1	R
2738	elkT-abcA_1_1	F
2739	elkT-abcA_1_1	R
2740	I-cadA_1_1	F
2741	I-cadA_1_1	R
2742	albA_1_1	F

SEQ ID NO	Probe name	Direction
2743	albA_1_1	R
2744	wzm_1_1	F
2745	wzm_1_1	R
2746	msrCb_1_1	F
2747	msrCb_1_1	R
2748	nov_1_1	F
2749	nov_1_1	R
2750	wzt_1_1	F
2751	wzt_1_1	R
2752	wbbl_1_1	F
2753	wbbl_1_1	R
2754	norA23_1_1	F
2755	norA23_1_1	R
2756	mexR_1_1	F
2757	mexR_1_1	R
2758	arr2_1_1	F
2759	arr2_1_1	R
2760	mreA_1_1	F
2761	mreA_1_1	R
2762	I-cadC_1_1	F
2763	I-cadC_1_1	R
2764	uvrA_1_1	F
2765	uvrA_1_1	R
2766	CRD2_1_1	F
2767	CRD2_1_1	R
2768	CDR1_1_1	F
2769	CDR1_1_1	R
2770	CDR1_2_1	F
2771	CDR1_2_1	R
2772	MET3_1_1	F
2773	MET3_1_1	R
2774	FET3_1_1	F
2775	FET3_1_1	R
2776	FTR2_1_1	F
2777	FTR2_1_1	R
2778	MDR1-7_1_1	F
2779	MDR1-7_1_1	R
2780	ERG11_1_1	F
2781	ERG11_1_1	R
2782	SEC20_1_1	F
2783	SEC20_1_1	R
2784	rbcL_1_1	F
2785	rbcL_1_1	R
2786	LDHA(hu)_1_1	F

SEQ ID NO	Probe name	Direction
2787	LDHA(hu)_1_1	R
2788	GAPD(hu)_1_1	F
2789	GAPD(hu)_1_1	R
2790	b-Act(hu)_1_1	F
2791	b-Act(hu)_1_1	R
2792	ARHGDIA(hu)_1_1	F
2793	ARHGDIA(hu)_1_1	R
2794	PGK1(hu)_1_1	F
2795	PGK1(hu)_1_1	R
2796	rbcL_1_2	F
2797	rbcL_1_2	R
2798	16SPa_1_1	F
2799	16SPa_1_1	R
2800	23SEfaecium_2_1	F
2801	23SEfaecium_2_1	R
2802	16SStrepyog_1_1	F
2803	16SStrepyog_1_1	R
2804	16SStrepneu_1_1	F
2805	16SStrepneu_1_1	R
2806	16SStrepagalactiae_1_1	F
2807	16SStrepagalactiae_1_1	R
2808	16SEfaecium_1_1	F
2809	16SEfaecium_1_1	R
2810	16SEfaecium_2_1	F
2811	16SEfaecium_2_1	R
2812	16SRNAEf_2_1	F
2813	16SRNAEf_2_1	R
2814	16SKpn_1_1	F
2815	16SKpn_1_1	R
2816	16SSa_3_1	F
2817	16SSa_3_1	R
2818	16SRNAEf_1_1	F
2819	16SRNAEf_1_1	R
2820	16SShominis_1_1	F
2821	16SShominis_1_1	R
2822	16SShaemolyt_1_1	F
2823	16SShaemolyt_1_1	R
2824	23SEfaecium_1_1	F
2825	23SEfaecium_1_1	R
2826	16SrRNAPrmi_1_1	F
2827	16SrRNAPrmi_1_1	R
2828	16SrRNAPrvu1_1_1	F
2829	16SrRNAPrvu1_1_1	R
2830	16SSa_1_1	F

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SEQ ID NO	Probe name	Direction
2831	16SSa_1_1	R
2832	16SKlox_1_1	F
2833	16SKlox_1_1	R
2834	p53_1_1	F
2835	p53_1_1	R
2836	0135mihck_1_1	F
2837	0135mihck_1_1	R
2838	FAN_1_1	F
2839	FAN_1_1	R
2840	0270cap_1_1	F
2841	0270cap_1_1	R
2909	16SStrepdysgal_1_1	F
2910	16SStrepdysgal_1_1	R
2911	carO_1_1	F
2912	carO_1_1	R
2913	gacS_1_1	F
2914	gacS_1_1	R
2915	dhbA_1_1	F
2916	dhbA_1_1	R
2917	dhbB_1_1	F
2918	dhbB_1_1	R
2919	sid_1_1	F
2920	sid_1_1	R
2921	csuD_1_1	F
2922	csuD_1_1	R
2923	csuC_1_1	F
2924	csuC_1_1	R
2925	tnp-ACIBA_1_1	F
2926	tnp-ACIBA_1_1	R
2927	waaA-ACIBA_1_1	F
2928	waaA-ACIBA_1_1	R
2929	csuB_1_1	F
2930	csuB_1_1	R
2931	csuA_B_1_1	F
2932	csuA_B_1_1	R
2933	csuA_1_1	F
2934	csuA_1_1	R
2935	put1_1_1	F
2936	put1_1_1	R
2937	por_1_1	F
2938	por_1_1	R
2939	abc_1_1	F
2940	abc_1_1	R
2941	furACIBA_1_1	F

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SEQ ID NO	Probe name	Direction
2942	furACIBA_1_1	R
2943	dec_1_1	F
2944	dec_1_1	R
2945	cysI_1_1	F
2946	cysI_1_1	R
2947	trpE_1_1	F
2948	trpE_1_1	R
2949	put3_1_1	F
2950	put3_1_1	R
2951	ompA-ACIBA_1_1	F
2952	ompA-ACIBA_1_1	R
2953	aacA4ENCL_1_1	F
2954	aacA4ENCL_1_1	R
2955	AdeR-ACIBA_1_1	F
2956	AdeR-ACIBA_1_1	R
2957	adeA-ACIBA_1_1	F
2958	adeA-ACIBA_1_1	R
2959	aac(6p)-lb7_1_1	F
2960	aac(6p)-lb7_1_1	R
2961	adeB-ACIBA_1_1	F
2962	adeB-ACIBA_1_1	R
2963	adeC-ACIBA_1_1	F
2964	adeC-ACIBA_1_1	R
2965	AdeS-ACIBA_1_1	F
2966	AdeS-ACIBA_1_1	R
2967	blaL2_1_1	F
2968	blaL2_1_1	R
2969	blaMIR-3_1_1	F
2970	blaMIR-3_1_1	R-
2971	ampR_1_1	F
2972	ampR_1_1	R
2973	ampC-ENCL_1_1	F
2974	ampC-ENCL_1_1	R
2975	blaL1_1_1	F
2976	blaL1_1_1	R
2977	asr_1_1	F
2978	asr_1_1	R
2979	lacZ_1_1	F
2980	lacZ_1_1	R
2981	ehuS_1_1	F
2982	ehuS_1_1	R
2983	ehuV_1_1	F
2984	ehuV_1_1	R
2985	slyA_1_1	F

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SEQ ID NO	Probe name	Direction
2986	slyA_1_1	R
2987	ORF165_1_1	F
2988	ORF165_1_1	R
2989	ehuU_1_1	F
2990	ehuU_1_1	R
2991	ehuT_1_1	F
2992	ehuT_1_1	R
2993	ORF295_1_1	F
2994	ORF295_1_1	R
2995	ehuA_1_1	F
2996	ehuA_1_1	R
2997	ORF400_1_1	F
2998	ORF400_1_1	R
2999	H+ATPase_1_1	F
3000	H+ATPase_1_1	R
3001	sulII_1_1	F
3002	sulII_1_1	R
3003	smeE_1_1	F
3004	smeE_1_1	R
3005	eE_1_1	F
3006	eE_1_1	R
3007	StmPr1_1_1	F
3008	StmPr1_1_1	R
3009	eD_2_1	F
3010	eD_2_1	R
3011	ppi_1_1	F
3012	ppi_1_1	R
3013	pmp-STEMA_1_1	F
3014	pmp-STEMA_1_1	R
3015	pam_1_1	F
3016	pam_1_1	R
3017	ORF4-STEMA_1_1	F
3018	ORF4-STEMA_1_1	R
3019	ORF2-STEMA_1_1	F
3020	ORF2-STEMA_1_1	R
3021	et_1_1	F
3022	et_1_1	R
3023	eF_1_1	F
3024	eF_1_1	R
3025	 StmPr2_1_1	F
3026	StmPr2_1_1	R
3027	smeF4494_1_1	F
3028	smeF4494_1_1	R
3029	coa_3_1	F

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SEQ ID NO	Probe name	Direction
3030	coa_3_1	R
3031	coa_2_2	F
3032	coa_2_2	R
3033	fasCAXStrdysg_1_1	F
3034	fasCAXStrdysg_1_1	R
3035	sloStrep_1_1	F
3036	sloStrep_1_1	R
3037	ydhK_1_1	F
3038	ydhK_1_1	R
3039	tetA-ACIBA_1_1	F
3040	tetA-ACIBA_1_1	R
3041	tetR-ACIBA_1_1	F
3042	tetR-ACIBA_1_1	R

## Claims

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- 1. An analytical device for direct identification and characterisation of microorganisms in a sample or clinical specimen, wherein the analytical device comprises species specific gene probes which are (i) selected from DNA sequences or partial DNA sequences of the microorganisms to be identified or DNA sequences complementary or homologous thereto, and (ii) have a length of at least 100 nucleotides (nt).
- 2. The analytical device of claim 1, which is a DNA coated bead, a set of DNA coated beads, or a DNA microarray, preferably a DNA microarray.
- 3. The analytical device of claim 1 or 2 which is suitable for species specific identification of one microbial strain or a plurality of microbial strains in clinical specimens comprising microbial strains, especially bacteria and/or fungi, and which furthermore allows differentiation of the target species from each other and from non-target-species contained in one sample comprising a plurality of microbial strains.
  - 4. The analytical device of claim 3 which is suitable for species specific identification of microorganisms causing bacteremia, fungemia or sepsis in a clinical sample.
  - 5. The analytical device of any one of claims 1 to 4, wherein the device is suitable for species specific identification of microorganisms selected from the group consisting of Staphylococci, *E. coli* and Candida sp., preferably for species specific identification of Staphylococci.
  - 6. The analytical device of any one of claims 1 to 5, which is suitable for species specific identification of microorganisms selected from the group consisting of Staphylococcus aureus, Escherichia coli, CoNS (including Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus warneri, Staphylococcus saprophyticus), Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Streptococcus agalactiae, Streptococcus mutans, Enterococcus faecalis, Enterococcus faecium, Proteus mirabilis, Proteus vulgaris, Candida albicans, Acinetobacter baumannii.
  - 7. The analytical device of claim 6, wherein the device is suitable for species specific identification of at least *S. aureus* and preferably comprises gene probes

- selected from SEQ ID NO:3-6, 31, 40, 50, 51, 58, 59, 63, 64, 66-69, 71, 74, 76, 77, 79, 2902 and 2903, more preferably from SEQ ID NO:4, 68, 69 and 71, even more preferably comprises at least SEQ ID NO:71.
- 8. The analytical device of claim 6 or 7, wherein the device is suitable for species specific identification of at least *S. aureus, E. coli,* CoNS, Enterococcus sp., and/or Candida sp., and preferably comprises gene probes selected from
  - a) SEQ ID NO:4, 68, 69 and 71, preferably SEQ ID NO: 71 for identification of *S. aureus*;
- b) SEQ ID NO: 145, 160, 161 and 170, preferably SEQ ID NO:145 for identification of *E. coli*;
  - c) SEQ ID NO:177, 178 and 190, preferably SEQ ID NO:178 for identification of *S. epidermidis*;
  - d) SEQ ID NO:60, 61, 70, 72, 78 and 125, preferably SEQ ID NO:78 for identification of the genus Staphylococci including *S. aureus*;
- e) SEQ ID NO:210, 224 and 2906, preferably 2906 for identification of CoNS;
  - f) SEQ ID NO:308, 310 and 314, preferably SEQ ID NO:310 for identification of Enterococcus faecalis;
  - g) SEQ ID NO:380 and 385, preferably SEQ ID NO:380 for identification of Enterococcus faecium;
- 20 h) SEQ ID NO:232 and 249, preferably SEQ ID NO:249 for identification of *Candida albicans*;

respectively.

- 9. The analytical device of claim 8, which is suitable for species specific detection or differentiation of
- 25 (i) S. aureus and comprises SEQ ID NO:71;
  - (ii) CoNS and comprises SEQ ID NO:2906;
  - (iii) E. coli and comprises SEQ ID NO:145; and/or
  - (iv) Candida albicans and comprises SEQ ID NO:249.

- 10. The analytical device of any one of claims 7 to 9, which is suitable for additional species specific identification or differentiation of one or more of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Proteus vulgaris*.
- 5 11. The analytical device of any one of claims 1 to 10, which additionally comprises virulence and/or resistance gene probes.
  - 12. The analytical device of any one of claims 1 to 11, wherein
  - (i) the length of the gene probes is from 100 to 1000 nt, preferably from 200 to 800 nt; and/or
- (ii) specific gene probes are present for each specific microbial species or group of microorganisms to be identified or differentiated, which gene probes preferably are DNA sequences selected from the groups consisting of (a) species specific gene probes, (b) virulence gene probes and (c) resistance gene probes; and/or
- (iii) the sample is selected from whole blood, serum, urine, saliva, liquor, sputum,
   punktate, stool, pus, wound fluid, swabs, positive blood cultures, preferably is positive blood cultures; and/or
  - (iv) the device further comprises DNA sequences selected from the group (d) consisting of control gene probes coding for negative controls and positive controls.
  - 13. The analytical device of claim 3, which is suitable for diagnosis of

- (i) bacteremia, fungemia or sepsis, wherein the device preferably comprises probes for species specific identification of at least *S. aureus, E. coli*, CoNS, Enterococcus sp., and Candida sp.;
  - (ii) respiratory tract infections, wherein the device preferably comprises probes for species specific identification of at least Candida sp., *S. aureus* and *P. aeruginosa*; and/or
  - (iii) urinary tract infenctions, wherein the device preferably comprises probes for species specific identification of at least *E. coli*, Enterococci sp., Candida sp. and Proteus sp..
- 14. The analytical device of any one of claims 1 to 13, wherein the set of geneprobes preferably comprises gene probes selected from

(a) species specific gene probes for

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- (i) Staphylococcus aureus including gene probes derived from from clfA, clfB, coa, lytM, NAG, sodA, sodB, epiP-bsaP, geh, hemC, hemD, hsdS, lip, menC, nuc, SAV0431, SAV0440, SAV0441, spa, ebpS, fbpA, fib, fnbB, srtA, fnbA, femA, fmhB, fmhA;
- (ii) Escherichia coli including gene probes derived b1169, fliCb, nfrB, yacH, ycdS, yciQ, shuA;
- (iii) Staphylococcus epidermidis including gene probes derived from ardeSE0106, ardeSE0107, atlE, agrB, alphSE1368, gad, glucSE1191, icaB, mvaSSepid, nitreSE1972, nitreSE1974, nitreSE1975, oiamtSE1209, ORF1Sepid, ORF3bSepid, qacR, ureSE1865, ureSE1867;
- (iv) Staphylococcus haemolyticus including gene probes derived from femBShaemolyt, mvaDShaemolyt, mvaSShaemolyticus, RNApolsigm;
- (v) Staphylococcus lugdunensis including gene probes derived from agrB2Stalugd,15 agrC2Stalugd, slamStalugd;
  - (vi) Staphylococcus warneri including gene probes derived from msrw1Stwar, nukMStwar, proDStwar, proMStwar, sigrpoStwar, tnpStwar;
  - (vii) Staphylococcus saprophyticus including gene probes derived from RNApolsigmSsapro;
- 20 (viii) Staphylococcus hominis including gene probes derived from ydhK;
  - (ix) Candida albicans including gene probes derived from ARG56, ASL43f, BGL2, CCT8, CDC37, CEF3, CHS1, CHS2, CHS4, CHS5, CHT1, CHT2, CHT4, CSA1, 5triphosphatase, AAF1, ADH1, ALS1, ALS7, EDT1, ELF, ESS1, FAL1, GAP1, GNA1, GSC1, GSL1, HIS1, HTS1, HWP1, HYR1, INT1a, KRE15f, KRE6, KRE9, MIG1, MLS1, MP65, NDE1, PFK2, PHR1, PHR2, PHR3, PRA1, PRS1, RBT1, RBT4, RHO1, RNR1, RPB7, RPL13, RVS167, SHA3, SKN1, SRB1, TCA1, TRP1, YAE1, YRB1, YST1exon2;
  - (x) Enterococcus faecalis including gene probes derived from arcA, arcC, bkdA, camE1, csrA, dacA, dfr, dhoD1a, ABC-eltA, agrBfs, agrCfs, dnaE, ebsA, ebsB, eep, efaR, gls24\_glsB, gph, gyrAEf, metEf, mntHCb2, mob2, mvaD, mvaE, parC, pcfG, phoZ, polC, ptb, recS1, rpoN, tms, tyrDC, tyrS;

- (xi) Enterococcus faecium including gene probes derived from bglB, bglR, bglS, efmA, efmB, efmC, mreC, mreD, mvaDEfaecium, mvaEEfaecium, mvaK1Efaecium, mvaK1Efaecium, mvaK2Efaecium, mvaSEfaecium, orf3\_4Efaeciumb, orf6\_7Efaecium, orf7\_8Efaecium, orf9\_10Efaecium;
- 5 (xii) Klebsiella pneumonia including gene probes derived from atsA, budC, citA, citW, citX, dalK, acoA, acoB, acoC, ahlK, fimK, glfKPN2, ltrA, mdcC, mdcH, , nifF, nifK, nifN, tyrP, wbbO, wzb, wzmKPN2, wztKPN2, yojH, liac;
  - (xiii) *Klebsiella oxytoca* including gene probes derived from *gatY*, *pelX*, *tagH*, *tagK*, *tagT*;
- 10 (xvi) Pseudomonas aeruginosa including gene probes derived from glpR, lasRb, OrfX, pa0260, pa0572, pa0625, pa0636, pa1046, pa1069, pa1846, pa3866, pa4082, pilAp, PilAp2, pilC, PstP, uvrDII, vsmI, vsmR, xcpX;
- (xv) Streptococcus pneumoniae including gene probes derived from cap1EStrpneu, cap1FStrpneu, cap1GStrpneu, cap3AStrpneu, cap3BStrpneu, celAStrpneu, 15 celBStrpneu, cqlAStrpneu, cqlBStrpneu, cqlCStrpneu, cqlDStrpneu, cinA, cps14EStrpneum, cps14FStrpneum, cps14GStrpneum, cps14HStrpneum, cps19aHStrpneum, cps19aIStrpneum, cps19aKStrpneum, cps19fGStrpneum, dinF, 1760Strpneu, acyPStrpneu, cps23fGStrpneum, dexB, endAStrpneu, exoAStrpneu, exp72, fnlAStrpneu, fnlBStrpneu, fnlCStrpneu, gct18Strpneum, 20 hftsHstrpneu, immunofrag1Strpneu, immunofrag2Strpneu, immunofrag3Strpneu, kdtBStrpneu, lysAStrpneu, pcpBStrpneu, pflCStrpneu, plpA, prtA1Strpneu, pspC1Strpneu, pspC2, purRStrpneu, pyrDAStrpneum, SP0833Strpneu, SP0828Strpneu, SP0830Strpneu, SP0837\_38Strpneu, SP0839Strpneu, ugdStrpneu, uncC, vicXStrepneu, wchA6bStrpneum, 25 wci4Strpneum, wciK4Strpneum, wciL4Strpneum, wciN6bStrpneum, Strpneum, wciP6bStrpneum, wciY18Strpneum, wzdbStrpneum, wze6bStrpneum, wzy18Strpneum, wzy4Strpneum, wzy6bStrpneum, xpt;
  - (xvi) Streptococcus agalactiae including gene probes derived from cpsA1Strgal, cpsB1Strgal, cpsC1Strgal, cpsD1Strgal, cpsE1Strgal, cpsG1Strgal, cpsIStragal, cpsIStragal, cpsYStragal, cylBStraga, cylEStraga, cylFStraga, cylHStraga, cylIStraga, cylJStraga, cylKStraga, 0483Straga, 0493Straga, 0495Straga, 0498Straga, 0500Straga, 0502Straga,

0504Straga, folDStraga, neuA1Strgal, neuB1Strgal, neuC1Strgal, neuD1Strgal, recNStraga, ileSStraga;

- (xvii) Streptococcus pyogenes including gene probes derived from cyclStrpyog, fah\_rph\_hlo\_Strpyog, int, int315.5, oppD, , SpyM3\_1351, vicXStrpyog;
- 5 (xviii) Streptococcus mutans including gene probes derived from 573Stprmut, 580SStprmut, 581\_582SStprmut, 584SStprmut, dltAStrmut, dltBStrmut, dltCppx1Strmut, dltDStrmut, lichStrbov, lytRStprmut, lytSStprmut, pepQStrrmut, pflCStrmut, recNStprmut, ytqBStrmut;
- (xix) Proteus mirabilis including gene probes derived from atfA, atfB, atfC, 10 ccmPrmi1, cyaPrmi, flfB, flfD, flfN, flhD, floA, ftsK, gstB, hemCPrmi, hemDPrmi, hev, katA, lpp1, menE, mfd, nrpA, nrpB, nrpG, nrpS, nrpT, nrpU, pat, pmfA, pmfC, pmfE, ppaA, rsbA, rsbC, speB, stmA, stmB, terA, umoA, umoB, umoC, ureR, xerC, ygbA;
- (xx) *Proteus vulgaris* including gene probes derived from *envZPrvu*, *frdC*, *frdD*, *lad*, 15 tna2;
  - (xxi) Acinetobacter baumanii including gene probes derived from carO, gacS, dhbA, dhbB, sid, csuD, csuC, tnp-ACIBA, waaA-ACIBA, csuB, csuA\_B, csuA, put1, por, abc, furACIBA, dec, cysI, trpE, put3, ompA-ACIBA; and/or
  - (b) virulence gene probes for
- (i) Staphylococcus aureus including gene probes derived from bsaE, bsaG, cap5h, cap5i, cap5j, cap5k, cap8H, cap8I, cap8J, cap8K, I-hld, I-hysA, I-IgGbg, EDIN, eta, etb, hglA, hglB, hglC, hla, hlb, lukF, lukS, NAG, sak, sea, seb, sec1, seg, seh, sel, set15, set6, set7, set8, sprV8, tst, I-sdrC, I-sdrD, I-sdrE;
- (ii) Escherichia coli including gene probes derived from b1202, eae, eltB, escR,
   escT, escU, espB, fes, fteA, hlyA, hlyB, iucA, iucB, iucC, papG, rfbE, shuA, SLTII, toxA-LTPA, VT2vaB;
  - (iii) Staphylococcus epidermidis including gene probes derived from gcaD, hld\_orf5, icaC, icaD, icaR, psm\_beta1and2, purR, spoVG, yabJ;
  - (iv) Staphylococcus haemolyticus including gene probes derived from lipShaemolyt;
- 30 (v) Staphylococcus lugdunensis including gene probes derived from fblStalugd, slushABCStalugd;

- (vi) Staphylococcus warneri including gene probes derived from gehAStwar;
- (vii) Candida albicans including gene probes derived from CCN1, CDC28, CLN2, CPH1, CYB1, EFG1, MNT1, RBF1, RBF1, RIM101, RIM8, SEC14, SEC4, TUP1, YPT1, ZNF1CZF1:
- 5 (viii) Enterococcus faecalis including gene probes derived from asa1, asp1, cgh, cylA, cylB, cylI, cylL\_cylS, cylM, ace, ef00108, ef00109, ef0011, ef00113, ef0012, ef0022, ef0031, ef0032, ef0040, ef0058, enlA, esa, esp, gelE, groEL, groES, rt1, sala, salb, sea1, sep1, vicK, yycH, yycI, yycJ;
- (ix) Enterococcus faecium including gene probes derived from entA\_entI, entD,10 entR, oep, sagA;
  - (x) Klebsiella pneumoniae including gene probes derived from cim, aldA, hemly, pSL017, pSL020, rcsA, rmlC, rmlD, waaG, wbbD, wbbM, wbbN, wbdA, wbdC, wztKpn, yibD;
- (xi) P. aeruginosa including gene probes derived from aprA, aprE, ctx, algB, algN,
   algR, ExoS, fpvA, lasRa, lipA, lipH, Orf159, Orf252, pchG, PhzA, PhzB, PLC, plcN,
   plcR, pvdD, pvdF, pyocinS1, pyocinS1im, pyocinS2, pys2, rbf303, rhlA, rhlB, rhlR,
   TnAP41, toxA;
  - (xii) Streptococcus pneumoniae including gene probes derived from igaStrpneu, lytA, nanA, nanBStrpneu, pcpCStrpneu, ply, prtAStrpneu, pspA, SP0834Strpneu, sphtraStrpneu, wciJStrpneu, wziyStrpneu, wzxStrpneu;

- (xiii) Streptococcus agalactiae including gene probes derived from CAMPfactor, 0499Straga, hylStragal, lipStragal;
- (xiv) Streptococcus pyogenes including gene probes derived from DNaseIStrpyog, fba2Strpyog, fhuAStrpyog, fhuB1Strpyog, fhuDStrpyog, fhuGStrpyog, hylA, hylP,
   25 hylp2, oppB, ropB, scpAStrpyog, sloStrpyog, smez- Strpyog, sof, speA, speB2Strpyog, speCStrpyog, speJStrpyog, srtBStrpyog, srtCStrpyog, srtEStrpyog, srtFStrpyog, srtFStrpyog, srtTStrpyog, vicKStrpyog;
- (xvi) *Streptococcus mutans* including gene probes derived from *hlyXStrmut*, 30 *perMStrmut*;

- (xvii) *Proteus mirabilis* including gene probes derived from *flaA*, *laD*, *fliA*, *hpmA*, *hpmB*, *lpsPrmi*, *mrpA*, *mrpB*, *mrpC*, *mrpD*, *mrpE*, *mrpF*, *mrpG*, *mrpH*, *mrpI*, *mrpJ*, *patA*, *putA*, *uca*, *ureDPrmi*, *ureEPrmi*, *ureFPrmi*, *zapA*, *zapB*, *zapD*, *zapE*; and/or
- (c) resistance gene probes derived from genes coding for
- (i) beta-lactams resistance including gene probes derived from blaIMP-7, mecISepid, blaOXA-10, blaB, ampC, blaR, blaOXA-32, bla-CTX-M-22, pbp2aStrpneu, blaSHV-1, blaOXA-2, blaRShaemolyt, blaIMP-7, mecR, blaOXY, dacCStrpyog, femA, mecA, blaIShaemolyt, blavim, pbp2b, pbp2primeSepid, pbp2x, pbp3Saureuc, pbp4, pbp5Efaecium, pbpC, mecI, pbp1a, blaI, blaTEM-106, blaOXY-10 KLOX, ftsWEF, fmhB, cumA, blaPER-1, bla\_FOX-3, blaA, psrb, fmhA, mecR1Sepid, blaZ, blaOXA-1, fox-6, blaPrmi;
  - (ii) aminoglycosides resistance including gene probes derived from aacA\_aphDStwar, aacC1, aacC2, strB, aadA, aadB, aadD, aacA4, strA, aph-A3, aacC1, aacA4, aacA-aphD, I-spc, aphA3, ; aacA4ENCL, aac(6p)-lb7;
- 15 (iii) macrolides-lincosamines-streptogramins resistance including gene probes derived from ermC, linB, satSA, mdrSA, I-linA, ermB, ermA, satA, msrA, mphBM, mefA, mrx;
  - (iv) trimethoprim resistance including gene probes derived from dfrA, dfrStrpneu;
- (v) chloramphenicol resistance including gene probes derived from cat,20 catEfaecium, cmlA5;
  - (vi) tetracyclines resistance including gene probes derived from tetAJ, tetL, tetM;
  - (vii) glycopeptides resistance including gene probes derived from vanH(tn), vanA, vanHB2, vanR, vanRB2, vanS(tn), vanSB2, vanWB2, ddl, ble, vanXB2, vanY(tn), vanYB2, vanB, vanZ(tn), vanC-2, vanX(tn);
- (viii) multiple target resistance including gene probes derived from acrB, mexB, I-qacA, sulI, sul, cadBStalugd, mexA, acrR, emeA, acrA, rtn, abcXStrpmut, qacEdelta1, elkT-abcA, I-cadA, albA, wzm, msrCb, nov, wzt, wbbl, norA23, mexR, arr2, mreA, I-cadC, uvrA, , AdeR-ACIBA, adeA-ACIBA, adeB-ACIBA, adeC-ACIBA, AdeS-ACIBA;
- 30 (ix) fungicide resistance, especially *C. albicans* fungicide resistance, including gene probes derived from *CRD2*, *CDR1*, *MET3*, *FET3*, *FTR2*, *MDR1-7*, *ERG11*, *SEC20*.

- 15. The analytical device of any one of claims 1 to 14, wherein
- (i) the device comprises the minimal number of species specific gene probes of group (a) as defined in claim 12 or 14 which is sufficient for species identification, preferably the device comprises at least 2 different gene probes per target species of group (a); and/or
- (ii) the device comprises the minimal number of virulence gene probes of group (b) as defined in claim 12 or 14 which is sufficient for virulence determination, preferably at least 1 gene probe, more preferably at least 5 different gene probes per target species of group (b); and/or
- (iii) the device comprises the minimal number of resistance gene probes of group (c) as defined in claim 12 or 14 which is sufficient for determination of resistance, preferably at least 1 gene probe, more preferably at least 5 different gene probes of group (c); and/or
- (iv) the DNA sequences are selected from the group consisting of SEQ ID NOs 1 918 and 2842-2908, complementary sequences thereto, addition mutants, deletion mutants, substitution mutants and homologues thereof.
  - 16. The analytical device of claim 15, wherein

- (i) the gene probes of group (a) are selected from SEQ ID NO:SEQ ID NO:1-99, 142-152, 174-199, 209-214, 216-219, 222-229, 231-291, 308-342, 377-393, 399-431, 449-490, 523-591, 606-639, 645-656, 687-701, 706-749, 776-781, 2843-2863, 2902 and 2903;
- (ii) the gene probes of group (b) are selected from SEQ ID NO:100-141, 153-173, 200-208, 215, 220-221, 230, 292-307, 343-376, 394-398, 432-448, 491-522, 592-605, 640-644, 657-686, 702-705, 750-775 and 782-784; and/or
- 25 (iii) the gene probes of group (c) are selected from SEQ ID NO:785-918, 2864-2875, 2888 and 2907-2908, preferably from SEQ ID NO:785-909, 2864-2875, 2888 and 2907-2908.
  - 17. The analytical device of claim 15 or 16, which
- (I) is suitable for identification of *Staphylococcus aureus* and comprises one or more or all of the gene probes selected from SEQ ID NO:3-6, 31, 40, 50, 51, 58,

- 59, 63, 64, 66-69, 71, 74, 76, 77, 79, 2902, 2903, preferably comprises at least one of the gene probes represented by SEQ ID NO:71, 68, 4 and 69; and/or
- (II) is suitable for identification of *Escherichia coli* and comprises one or more or all of the gene probes selected from SEQ ID NO:142, 144, 145, 148, 150-152, 160, 161 and 170, preferably at least one of the gene probe represented by SEQ ID NO:145, 160, 161 and 170; and/or

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- (III) is suitable for identification of *Staphylococcus epidermidis* and comprises gene probes selected from SEQ ID NO:174, 175, 177, 178, 180-182, 185-193, 198 and 199, preferably at least one of the gene probes represented by SEQ ID NO:177, 178 and 190; and/or
- (IV) is suitable for identification of *Staphylococcus haemolyticus* and comprises one or more or all of the gene probes selected from SEQ ID NO:211, 213 and 214, preferably at least one of the gene probes represented by SEQ ID NO:211 and 214; and/or
- (V) is suitable for identification of Staphylococcus lugdunensis and comprises one or more or all of the gene probes selected from SEQ ID NO:216, 217 and 219-221, preferably at least one of the gene probes represented by SEQ ID NO:216, 219, 220 and 221; and/or
- (VI) is suitable for identification of *Staphylococcus warneri* and comprises one or more or all of the gene probes selected from SEQ ID NO:224-228 and 230, preferably at least one of the gene probes represented by SEQ ID NO:224, 226, and 230; and/or
  - (VII) is suitable for identification of *Staphylococcus saprophyticus* and comprises one or more or all of the gene probes selected from SEQ ID NO:222 and 223; and/or
  - (VIII) is suitable for identification of *Staphylococcus hominis* and comprises one or more or all of the gene probes selected from SEQ ID NO:2096, 194, 229, 211 and 214; and/or
- (IX) is suitable for identification of *Candida albicans* and comprises one or more or all of the gene probes selected from SEQ ID NO:231-291, preferably at least one of the gene probes represented by SEQ ID NO:232 and 249; and/or

- (X) is suitable for identification of *Enterococcus faecalis* and comprises one or more or all of the gene probes selected from SEQ ID NO:308-310 and 312-342, preferably at least one of the gene probes represented by SEQ ID NO:308, 310 and 314; and/or
- 5 (XI) is suitable for identification of *Enterococcus faecium* and comprises one or more or all of the gene probes selected from SEQ ID NO:377-393, preferably at least one of the gene probes represented by SEQ ID NO:380 and 385; and/or
  - (XII) is suitable for identification of *Klebsiella pneumoniae* and comprises one or more or all of the gene probes selected from SEQ ID NO:399, 401-404, 408-415, 417, 420-423, 425 and 427-431, preferably at least one of the gene probes represented by SEQ ID NO:401, 410 and 430; and/or

- (XIII) is suitable for identification of *Klebsiella oxytoca* and comprises one or more or all of the gene probes selected from SEQ ID NO:459 and 466-469, preferably at least one of the gene probes represented by SEQ ID NO:459, 468 and 469; and/or
- 15 (XIV) is suitable for identification of *Pseudomonas aeruginosa* and comprises one or more or all of the gene probes selected from SEQ ID NO:470-485, 487-493 and 505, preferably at least one of the gene probes represented by SEQ ID NO:471, 474, 488 and 505; and/or
  - (XV) is suitable for identification of *Streptococcus pneumoniae* and comprises one or more or all of the gene probes selected from SEQ ID NO:523-591, preferably at least one of the gene probes represented by SEQ ID NO:558 and 562; and/or
  - (XVI) is suitable for identification of *Streptococcus agalactiae* and comprises one or more or all of the gene probes selected from SEQ ID NO:606-639, preferably at least one of the gene probes represented by SEQ ID NO:606 and 619; and/or
- 25 (XVII) is suitable for identification of *Streptococcus pyogenes* and comprises one or more or all of the gene probes selected from SEQ ID NO:645-648, 652, 655-656, 658 and 660, preferably at least one of the gene probes represented by SEQ ID NO:645, 658 and 660; and/or
- (XVIII) is suitable for identification of *Streptococcus mutans* and comprises one or more or all of the gene probes selected from SEQ ID NO:687-701, preferably at least one of the gene probes represented by SEQ ID NO:687, 691 and 692; and/or

- (XIX) is suitable for identification of *Proteus mirabilis* and comprises one or more or all of the gene probes selected from SEQ ID NO:706-710, 712-742 and 744-749, preferably at least one of the gene probes represented by SEQ ID NO:721, 725 and 735; and/or
- 5 (XX) is suitable for identification of *Proteus vulgaris* and comprises one or more or all of the gene probes selected from SEQ ID NO:776-778 and 780-781, preferably at least one of the gene probes represented by SEQ ID NO:776, 777 and 781; and/or
- (XXI) is suitable for identification of *Acinetobacter baumannii* and comprises one or more or all of the gene probes selected from SEQ ID NO:2843-2863, preferably at least one of the gene probes represented by SEQ ID NO:2858 and 2863.
  - 18. The analytical device of claim 17, which further comprises

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- (I) for the characterisation of *Staphylococcus aureus*: one or more or all of the gene probes of group (b) selected from SEQ ID NO:100-141, and/or
- of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
  - (II) for the characterisation of *Escherichia coli*: one or more or all of the gene probes of group (b) selected from SEQ ID NO:153-173, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
  - (III) for the characterisation of *Staphylococcus epidermidis:* one or more or all of the gene probes of group (b) selected from SEQ ID NO:200-208, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
- 25 (IV) for the characterisation of *Staphylococcus haemolyticus*: one or more or all of the gene probe of group (b) represented by SEQ ID NO:215, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
  - (V) for the characterisation of Staphylococcus lugdunensis: one or more or all

of the gene probes of group (b) selected from SEQ ID NO:220-221, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(VI) for the characterisation of *Staphylococcus warneri*: one or more or all of the gene probe of group (b) represented by SEQ ID NO:230, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

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- (VII) for the characterisation of *Staphylococcus saprophyticus*: one or more or all of the gene probe of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
- (VIII) for the characterisation of *Staphylococcus hominis*: one or more or all of the gene probe of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
- (IX) for the characterisation of *Candida albicans*: one or more or all
  of the gene probes of group (b) selected from SEQ ID NO:292-307, and/or of the gene probes of group (c) selected from SEQ ID NO:910-918; and/or
  (X) for the characterisation of *Enterococcus faecalis*: one or more or all of the gene probes of group (b) selected from SEQ ID NO:343-376, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
  - (XI) for the characterisation of *Enterococcus faecium:* one or more or all of the gene probes of group (b) selected from SEQ ID NO:394-398, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
- 25 (XII) for the characterisation of *Klebsiella pneumonia*: one or more or all of the gene probes of group (b) selected from SEQ ID NO:432-448, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(XIII) for the characterisation of *Klebsiella oxytoca:* one or more or all of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(XIV) for the characterisation of *Pseudomonas aeruginosa:* one or more or all of the gene probes of group (b) selected from SEQ ID NO:491-522, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(XV) for the characterisation of *Streptococcus pneumoniae*: one or more or all of the gene probes of group (b) selected from SEQ ID NO:592-605, and/or

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of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(XVI) for the characterisation of *Streptococcus agalactiae:* one or more or all of the gene probes of group (b) selected from SEQ ID NO:640-644, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

(XVII) for the characterisation of *Streptococcus pyogenes:* one or more or all of the gene probes of group (b) selected from SEQ ID NO:657-686, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or

- 20 (XVIII) for the characterisation of Streptococcus viridans: one or more or all of the gene probes of group (b) selected from SEQ ID NO:702-705, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
- of the gene probes of group (b) selected from SEQ ID NO:750-775, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908; and/or
  - (XX) for the characterisation of Proteus vulgaris: one or more or all

(XIX) for the characterisation of Proteus mirabilis: one or more or all

of the gene probes of group (b) selected from SEQ ID NO:782-784, and/or of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908.

- (XXI) for the characterisation of Acinetobacter baumannii: one or more or all
- of the gene probes of group (c) selected from SEQ ID NO:785-909, 2864-2875, 2888, 2907-2908.
  - 19. Use of the analytical device of any one of claims 1-18 for *in vitro* identification and characterisation of microorganisms in a sample or in a clinical specimen, preferably for the diagnosis of a clinical condition, more preferably for the diagnosis of bacteremia, fungemia or sepsis.
  - 20. Use of the analytical device of any one of claims 1-18 for *in vitro* differentiation of a plurality of different microbial strains contained in one sample and/or for species-specific identification of one or more microbial strain contained in a mixture of a plurality of microorganisms.
- 15 21. An *in vitro* method for identification and characterisation of microorganisms in a sample or in a clinical specimen comprising
  - (a) isolating the total DNA from the sample or clinical specimen and labelling the DNA with a reporter molecule;
  - (b) applying the DNA thus obtained to the analytical device of anyone of claims 1-18 and hybridising the DNA with the gene probes of the analytical device; and
    - (c) detecting DNA bound to the analytical device by determination of the amount of the reporter molecules bound to the device.
    - 22. The method of claim 21,

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- (i) which is a method for diagnosis of bacteremia, fungemia or sepsis; and/or
- 25 (ii) wherein the clinical specimen is a positive blood culture; and/or
  - (iii) wherein the ratio of microbial DNA to total DNA isolated from said sample or clinical specimen is less than 100 %, preferably from 1% to 99%; and/or
  - (iv) wherein the reporter molecule is a fluorochrome; and/or
- (v) wherein the determination of the amount of reporter molecules bound to the
   device is achieved by visualization of the reporter molecule; and/or

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- 220 -

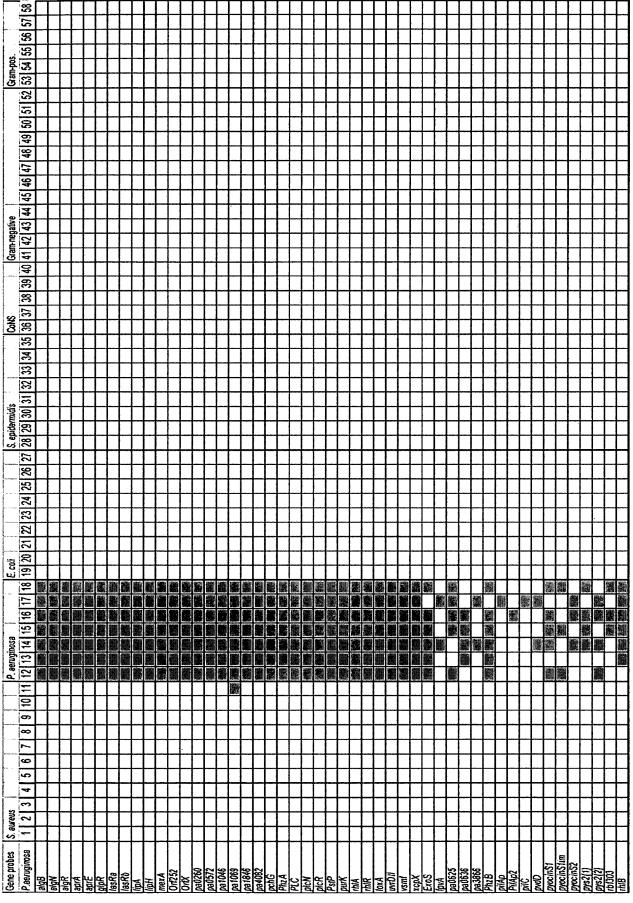
- (vi) wherein the DNA isolated in step (a) is labelled and applied to the analytical device without prior amplification, preferably is labelled by random priming; and/or
- (vii) wherein the DNA isolated in step (a) is fragmented before the labelling reaction.
- 5 23. The method of claim 21 or 22, wherein the analytical device is a DNA microarray and the detection is preferably performed using a DNA microarray reader.
  - 24. The method of claim 21 or 22, wherein the analytical device is a DNA coated bead or a set of DNA coated beads, and the application and/or detection step is preferably performed in a microfluidic device.

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25. A kit for detection of microorgamisms in a sample or clinical specimen comprising the analytical device of any one of claims 1 to 18.

Fig. 14

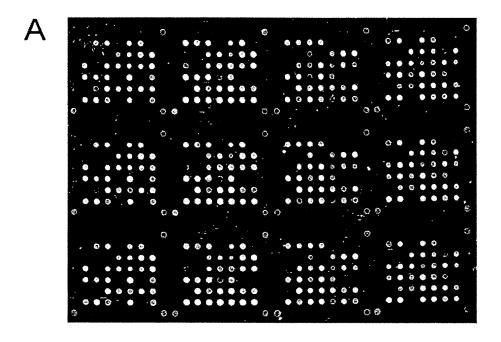
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Fig. 10



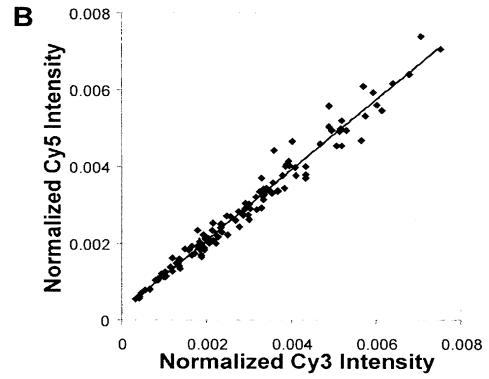


Fig.2

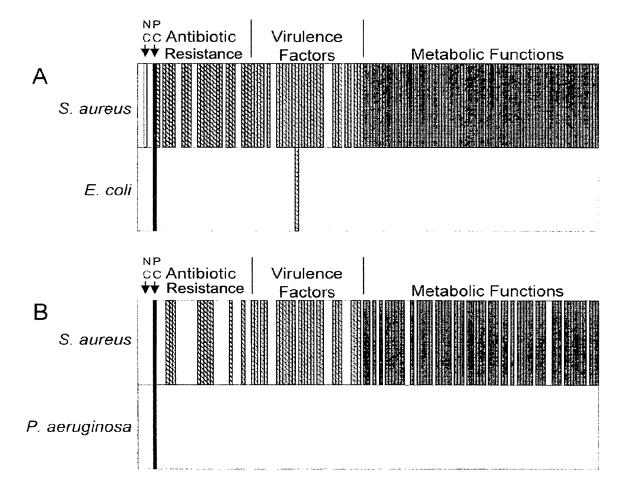


Fig.3

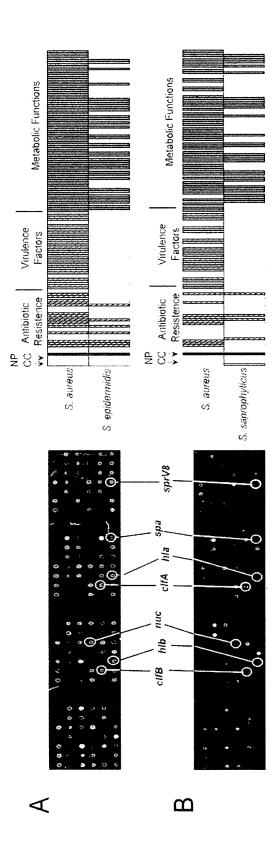
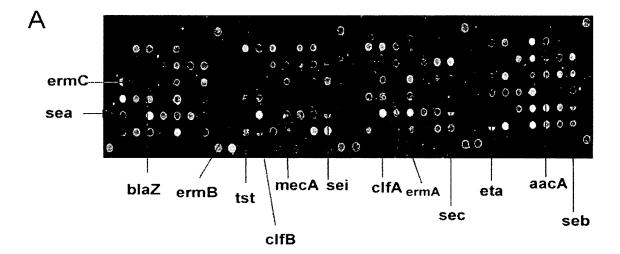


Fig.4



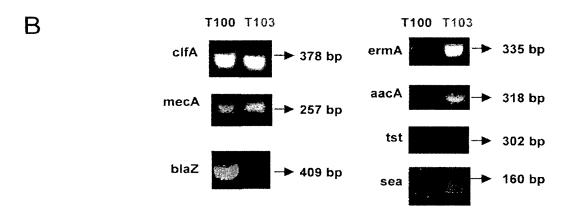
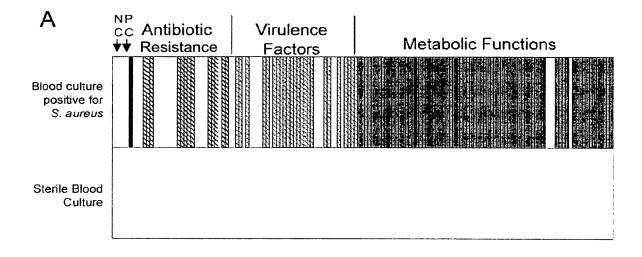


Fig.5



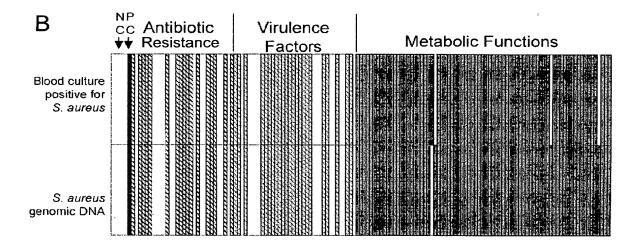


Fig.6

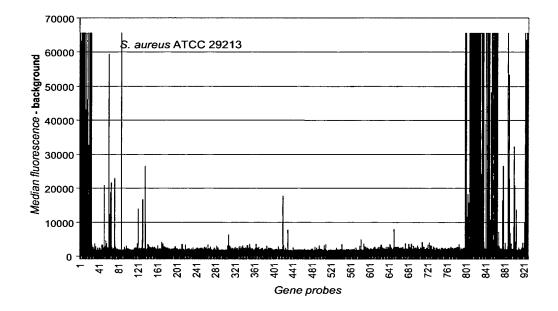


Fig.7A

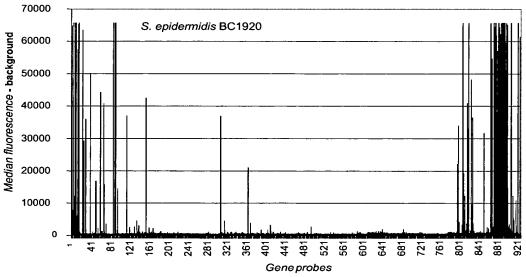
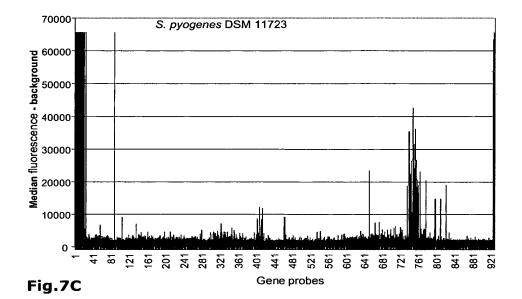


Fig.7B



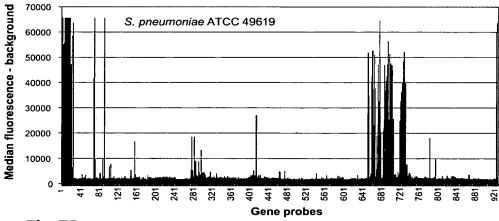
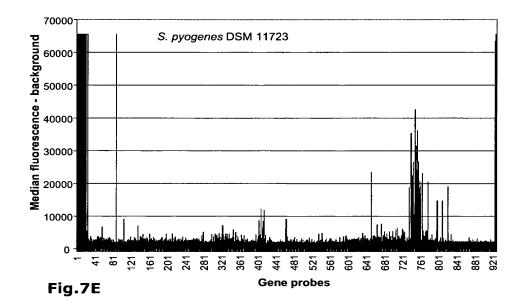


Fig.7D



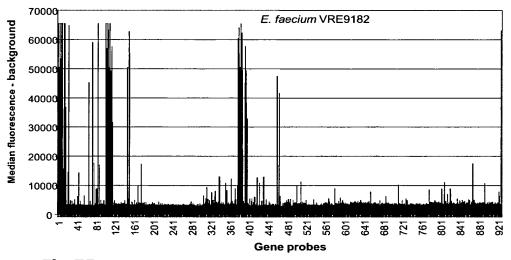


Fig.7F

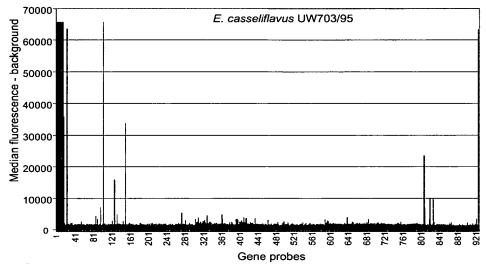


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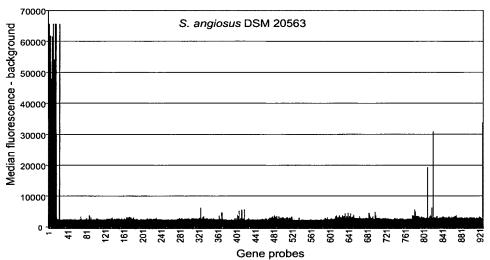


Fig.7H

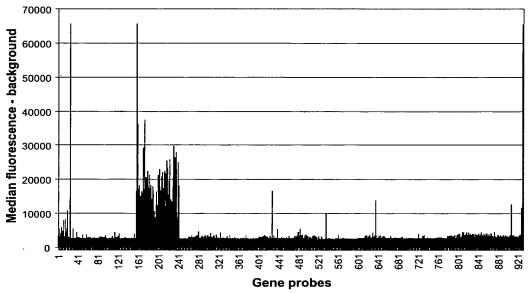
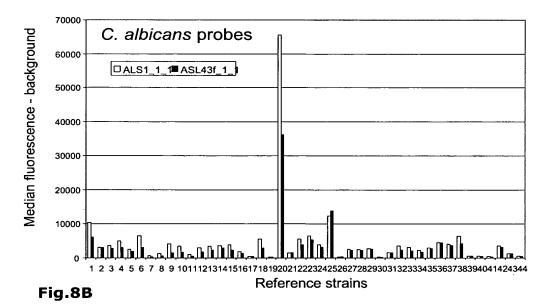


Fig.8A



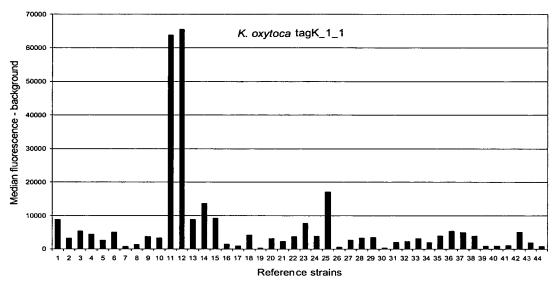


Fig.9A

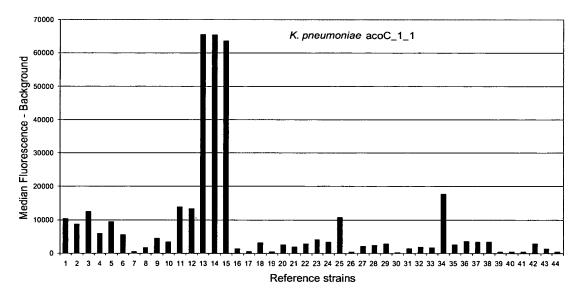


Fig.9B

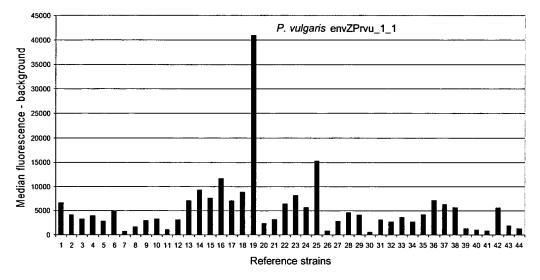


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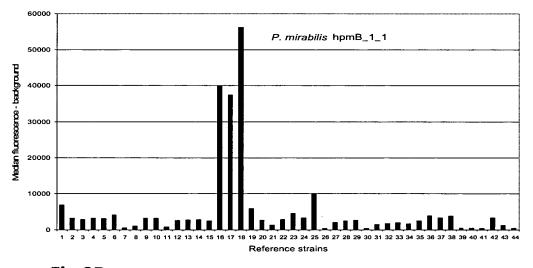
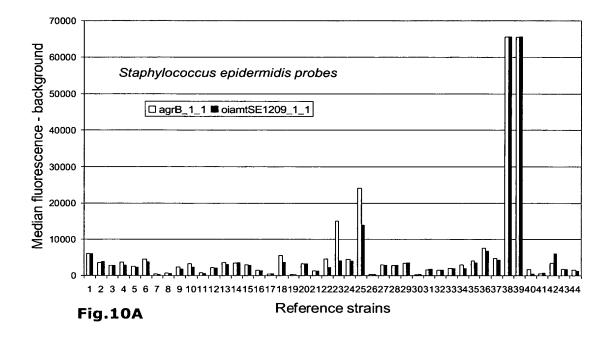
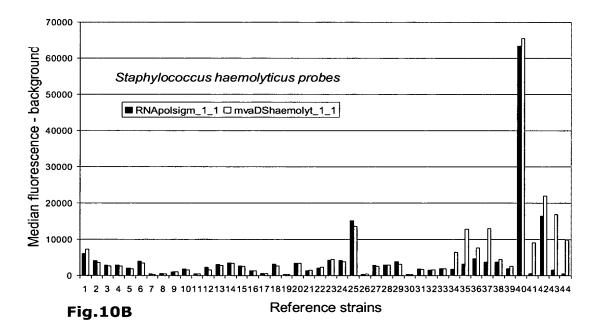
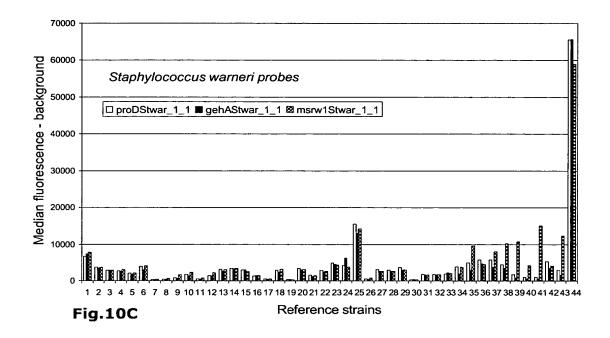


Fig.9D







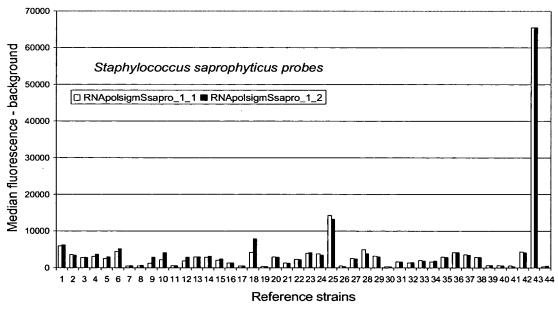


Fig.10D

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